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The effects and interactions of milk proteins on the properties of oat starch

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A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy in Food Science

at

Lincoln University

by

Lokesh Kumar

Lincoln University

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Declaration

Some parts of this thesis have been published, submitted and/or presented at Conferences in advance of submission of the thesis.

Publications

- Kumar, L., Brennan, M. A., Mason, S. L., Zheng, H., & Brennan, C. S. (2017). Rheological, pasting and microstructural studies of dairy protein-starch interactions and their application in extrusion-based products: A review. *Starch - Stärke*, *69*(1–2).

- Kumar, L., Brennan, M., Zheng, H., & Brennan, C. (2018). The effects of dairy ingredients on the pasting, textural, rheological, freeze-thaw properties and swelling behaviour of oat starch. *Food Chemistry*, *245*, 518–524.

- Kumar, L., Brennan, M., Zheng, H., & Brennan, C. (2018). The Effects of milk protein ingredients on pasting, hydration, syneresis and large deformation viscoelastic properties of oat flour. In submission.

Presentations

- Oat starch-milk protein ingredients interactions. An oral presentation at the 7th International Symposium on Delivery of Functionality in Complex Food Systems Conference, New Zealand in 2017.
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Project

- Some part of this thesis has been a part of commercial project named "Investigation of the interactions of multi-grain material with milk protein fractions to develop improved second generation extruded nutritional bars" conducted for Yili-Oceania Dairy industrial group Co., Ltd., China.

Abstract of a thesis submitted in partial fulfilment of the
requirements for the Degree of Philosophy in Food science.

The effects and interactions of milk proteins on the properties of oat starch

by

Lokesh Kumar

Oats are minor cereal crop and widely used as breakfast cereals. Oat starch is a by-product derived from the β-glucan extraction process and is widely used in food and non-food products such as oat meal, soups, gravies, coating agents for tablets and gloves and cosmetic products. It is also used for oat meal and other novel oat based food products such as oat puddings and oat-based sports gel consisting of oat starch and milk protein ingredients. These products normally undergo thermal treatment, where starch-milk protein interactions could affect the product's final rheological and sensory properties. Oat starch also exhibits unique properties including small granule size, a well developed granule surface and high lipid content. Hence, the overall aim of this thesis was to determine the effect of milk protein ingredients on oat starch and to advance our understanding of how starches and milk proteins interact with each other.

The effect of substituition of oat starch and oat flour at various concentrations (5% and 10%, w/w) with three different readily available milk protein ingredients, namley whey protein

concentrate (WPC), whey α-lactalbumin (WLAC) and skim milk powder (SMP) were investigated. All three ingredients decreased the pasting responses of oat starch. SMP showed a greater effect on oat starch characteristics in oat starch and flour systems in comaprison with WPC and WLAC. It was evident that even a small (5%) substituition of oat starch/flour with milk protein ingredients resulted in loss of native properties of oat starch/flour systems, where SMP affected these properties most. SMP increased the pasting temperature and decreased the starch swelling.

The effect of the addition of different concentrations (25%, 50%, 75% and 100%) of pure protein fractions, whey protein isolate (WPI) and calcium caseinate (CaCN) on oat starch were also investigated. Studies on the thermal behaviour of oat starch/milk proteins mixtures by differential scanning calorimetry (DSC) showed that WPI and CaCN increased the peak temperature (T_P), suggested delaying of starch gelatinisation, where WPI had a greater effect on oat starch than CACN. The X-ray diffraction and Fourier transform infrared spectroscopy studies revealed that oat starch/WPI mixtures increased the relative crytallinity suggesting an increase in ordered structure, while a decrease in IR bands at 1024 cm^{-1} and 1080 cm^{-1} indicated less gelatinisation of starch molecules. CaCN did not affect the relative crystallinity and showed an increase in amorphous structuration in FTIR analysis, which is an indication of complete gelatinisation.

The qualitative viscoelastic behaviour of oat starch/milk proteins gels were studied in terms of steady state flow behaviour, amplitude strain sweep and thixotropic analyis. WPI and CaCN increased the shear thinning behaviour of oat starch/milk proteins gels, while WPI decreased the elastic modulus (G') up to 75% concentration and increased at 100%. However, CaCN resulted in an increase in viscous modulus (G"), indicating weak viscous gels in comparison

with WPI/ oat starch gels. WPI and CaCN at high concentration (100%) resulted in an increase in elastic nature, but gels were found to be brittle in nature.

Finally, it was concluded that WPI illustrated high potency to affect oat starch gelatinisation in comparison with CaCN. This study is a first step to understand the oat starch- milk protein ingredients behaviour during thermal processing, and the trends obtained from the resultant work could predict the usage of oat starch/milk proteins in the development of oat-milk based foods.

Keywords: Oat starch, whey protein, calcium caseinate, skim milk powder, pasting properties, gelatinisation, rheological properties, thermal properties, Differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), amplitude strain-sweep test, time dependent viscosity, scanning electron microscopy (SEM), steady state, dynamic rheology, weltman model

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Abbreviations

3ITT- Three interval thixotropy test
A- Initial shear stress
a*- Redness
A_a- Amorphous area
A_c- Crystalline Area
ACE- Angiotensin-converting-enzyme
ANOVA- Analysis of Variance
AP- Amylopectin
B- time coefficient of thixotropic breakdown
b*- Yellowness
BSA- Bovine serum albumin
C- Casein
CaCN- Calcium caseinate
CLSM- Confocal laser scanning microscopy
CWM- Cross-linked waxy maize
DSC- Differential scanning calorimetry
FTIR- Fourier transform infrared spectroscopy
G*- Complex modulus
G'- Storage Modulus
G"- Loss modulus
GOPOD- Glucose oxidase/peroxidase
IR- Infrared
K- Consistency coefficient
L*- Lightness
MPC- Milk protein concentrate
n- Flow behaviour index
PCS- Phosphate crosslinked starch
RC- Relative crystallinity
Rec_{60}- Recovery at 60 sec
RTE- Ready to eat
RVA- Rapid visco analyser
SDS-PAGE- Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM- Scanning electron microscope
SMP- Skim milk powder
SOL- Solubility
SP- Swelling Power
SR- Stability Ratio
t- time
TA- Texture analyser
tan δ- Damping factor

T_c- Conclusion temperature
TEM- Transmission electron microscopy
T_o- Onset temperature
T_{p-} Peak viscosity
WAI- Water absorption index
WBC- Water binding capacity
WCF- Water chestnut flour
WLAC- Whey lactalbumin
WSI- Water solubility index
WPI- Whey protein isolate
WPC- Whey protein concentrate
XRD- X-Ray diffraction
α-lac- α-lactalbumin
β-lac- β-lactoglobulin
γ- Shear rate
γ_{max}- Maximum strain
ΔE- Total color difference
ΔH- enthalpy of gelatinization
ΔT- Transition temperature
Δη- Difference between initial and final viscosity
η- Apparent viscosity
η_o- initial viscosity
σ- Shear stress
σ_{max}-Maximum shear stress
σ_o- Yield stress

Chapter 1

1 Introduction

Oats belong to the genus *Avena* and are considered a minor cereal crop based on annual production. Oats are mainly used as an animal feed but are gaining popularity as a breakfast cereal in forms like oat meal, ready-to-eat cereal and cereal bars. Oats are considered to be a good source of dietary fiber as β-glucan (Weightman, Heywood, Wade, & South, 2004). Oat starch exhibits unique granule properties including small granule size, a well-developed granule surface, and high lipid content (Hoover, Smith, Zhou, & Ratnayake, 2003; Mirmoghtadaie, Kadivar, & Shahedi, 2009). Starch is a polysaccharide with extensive applications in food technology, because of its texture enhancing properties, its high availability and low cost.

Milk has been classified into two type of proteins i.e. casein and whey, both protein have distinct functional properties and nutrition to human health. Whey proteins have many valuable functional properties such as gelation characteristic, water holding capacity, anti-oxidant properties, immunostimulatory, anti-carcinogenic, hypocholesterolaemic, gut lining maintenance, which makes them more adequate for industrial use than caseins especially when fortifying food products (Hoppe et al., 2008; Onwulata, Smith, Konstance, & Holsinger, 2001).

Use of starch in dairy based products is very popular and prominent because of its functional properties such as a thickener, gelling agent or fat replacer in products like yoghurt, processed cheese and desserts. The starch gels containing milk proteins might form a co-gel, where both the biopolymers could contribute in formation of a gel. Starch gelatinisation is a process of swelling of starch molecules in the presence of water with constant heat, and results in the

breaking down of starch crystalline structure, allowing for the release of amylose and amylopectin into the system leading to paste, which is viscoelastic liquid in nature. When paste is cooled, restructuration of three dimensional network results int a gel (Kumar, Brennan, Mason, Zheng, & Brennan, 2017). A gel is defined by viscoelastic solid nature and need some initial force before viscous movement.

It is interesting to understand the starch-protein interactive effect where they are used together in a product, and how that affecst the overall rheological, pasting, gelatinisation, textural and physio-chemical properties of the food system. There are various studies on the properties of different cereal starches and dairy and non-dairy proteins separately, but quite a few studies on the interaction of milk proteins and oat starch. The starch-protein interactions were discussed by previous researchers and many possible hypotheses have been proposed in their literature; however the actual mechanism of interactions and fundamental aspects remain unclear (Kett et al., 2013; Ravindra, Genovese, Foegeding, & Rao, 2004; Vu Dang, Loisel, Desrumaux, & Doublier, 2009; H. Yang, Irudayaraj, Otgonchimeg, & Walsh, 2004).

Therefore, in systems in which milk proteins and starch co-exist, it is important to understand the mechanisms, interactions and synergistic effects that can provide maximum benefit to a food product. The aim of this research plan was to study different interactions and functionality of milk proteins with oat starch and oat flour. This study was aimed to understand how milk proteins affect oat starch in terms of substitution and addition of starch with milk. The substitution of oat starch with readily available milk protein ingredients was opted to study because the substitution of oat starch could lead to less glycemic index/starch digestibility and can also contribute to more protein content (Yong, Chan, Garcia, & Sopade, 2011). The other aspect was chosen to see the effect of proteins, when starch concentration kept constant, where availability of water could affect the gelatinisation. This study will put an

emphasis on the recent development in the field of milk protein-starch interactions in native food systems. The information covered in this research will be useful for novel food texture development using milk proteins and starches and to understand the interaction mechanism between starch and milk proteins, and the usage of protein-starch complex systems in various food formulations as well as to identify future research opportunities in this area. This knowledge will be useful to manufacturers who may seek to enhance food texture by blending different proteins. The resultant studies could explain the characteristics and behaviour of these interactions in various environments that will be helpful for various dairy and cereal products manufacturing industries as bakery products, breakfast foods, dairy drinks and RTEs etc. during processing.

1.1 Aim and objectives of the research

1.1.1 Aim of the research

The aim of this research work is to study the effects and interactions of different milk protein ingredients with oat starch and flour systems. The research is mainly divided into two aspects: First, the extraction of oat starch from oat flour and effect of readily available commercial milk protein ingredients (WPC, WLAC and SMP) on oat starch and oat flour systems.

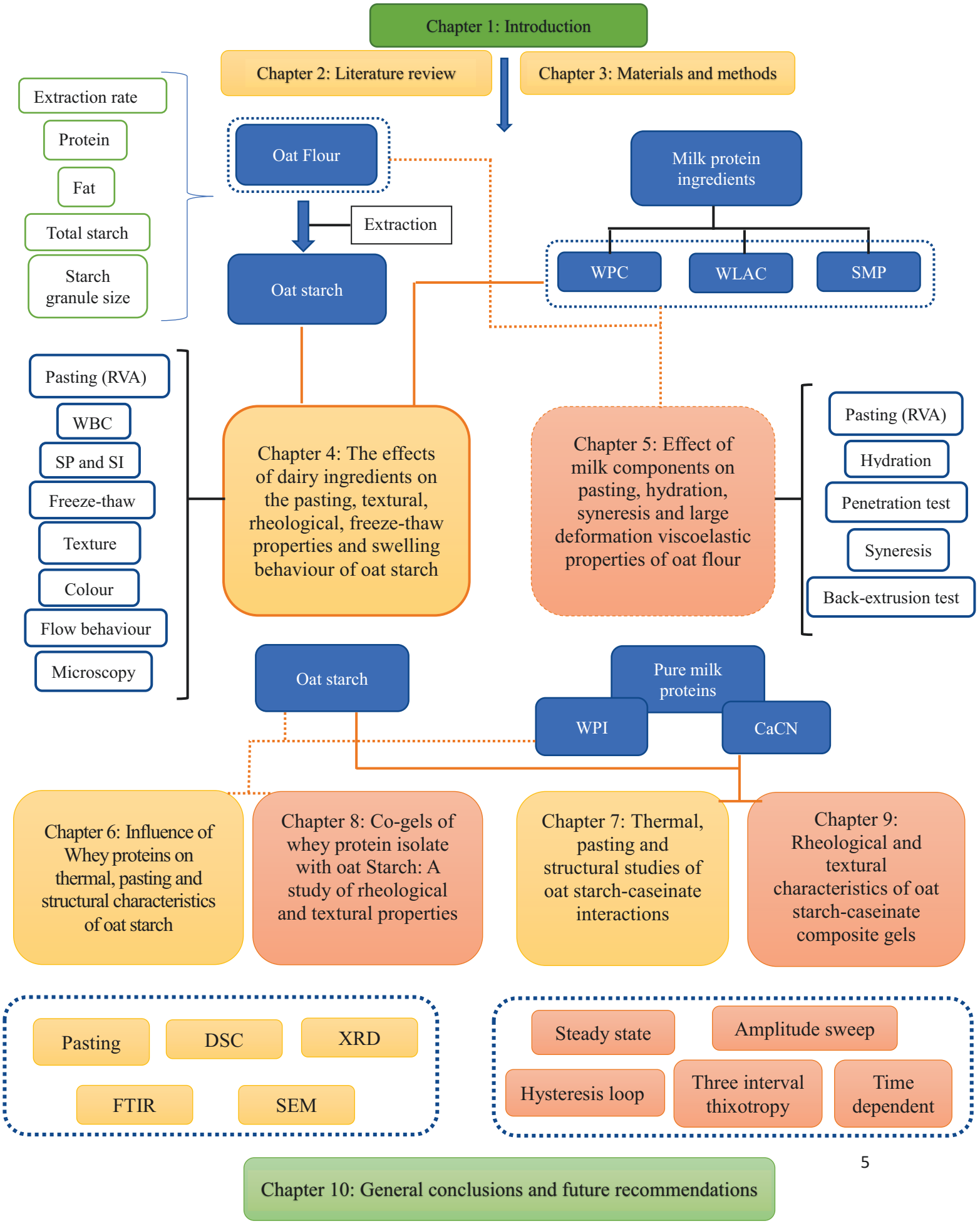
Second, the effects of more pure fractions of milk proteins (WPI and CaCN) on oat starch. This research aimed to illustrate the effect of milk proteins on oat starch, oat starch unique starch granule, which is smaller in comparison with other cereal starches and consist of high amount of lipids, could behave differently than other cereal starches. There is limited literature found that illustrates oat starch and milk protein interactions. It can be hypothesized that whey and casein protein will affect the gelatinisation properties of oat starch, while whey/casein-starch network will show synergistic effects in starch gels

1.2 Objectives of the research

In order to achieve this broad aim, the framework of this investigation is developed by classify the research work into following main task areas:

1. Isolation and characterization of oat starch from oat flour.
2. To study the effect of oat starch substitution with t milk protein.
3. To study the effect of oat flour substitution with milk protein ingredients on oat flour properties.
4. To Investigate the effect of addition of whey protein isolate (WPI) in oat starch system in terms of rheological, thermal, pasting and structural properties.
5. To investigate the effect of calcium caseinate (CaCN) addition in oat starch system to predict its effect on rheological, thermal, pasting and structural properties.

1.3 Thesis structure



Chapter 2

2 Review of Literature

- Some of this part has been published in Starch-Starke (Kumar, L., Brennan, M. A., Mason, S. L., Zheng, H., & Brennan, C. S. (2017). Rheological, pasting and microstructural studies of dairy protein-starch interactions and their application in extrusion-based products: A review. *Starch - Stärke*, *69*(1–2).

2.1 Oats (Avena sativa)

Oat is primarily a cool season crop. Cold climate countries such as northern and eastern Europe, northern USA, Canada and New Zealand have favourable conditions for oat cultivation (Miller & Fulcher, 2011). The popularity of oats as a part of human diet has increased because of nutritional properties of oat β-glucans (Nieto-Nieto, Wang, Ozimek & Chen, 2014).

The protein content of oat is much higher than that of other cereal grains, and oat oil has a favourable ratio of polyunsaturated to saturated lipid (Autio & Eliasson, 2009). Interest in increasing the utilization of oats for human consumption has been stimulated by the need for high soluble fiber in consumer's daily diet (Chen, Jiao, & Ka, 2008). The most common use for oats in human consumption is in the hot or roasted breakfast cereal due to its high β-glucan content which ranges from 2 to 8%, known for its ability to lower cholesterol (Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009; Zhu, 2017).

2.1.1 Oat structure

The raw oat kernel is composed of four major subunits: the hull, bran, endosperm and the germ (Figure 2-1). Normally, the hull is removed apart from the groat before use (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008). Then the oat groat, i.e. de-hulled oat grain, can be divided in to three major divisions the bran, endosperm and germ.

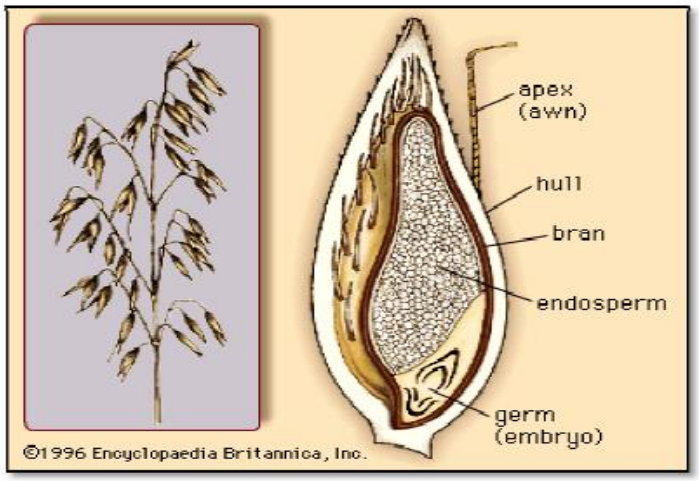


Figure 2-1 Oat grain structure

2.1.1.1 Hull

Around 25-30% weight of the oat grain is taken by the hull, which acts as a covering with numerous trichomes or hair like protuberance, and is normally separated from the oat groat before use in the food industry (Butt et al., 2008). However, for example in China, hull-less oats are commonly grown. During harvest the hull separates easily from the seed and no de-hulling process is required (Miller & Fulcher, 2011).

2.1.1.2 Bran

Oat bran is composed of different layers, like pericarp, testa (seed coat), nucellus, aleurone, sub aleurone and starchy endosperm (Figure 2-1). It is similar to other cereal bran, such as wheat bran, but the thickness and chemical composition of the layers are different (Surget & Barron, 2005). Oat bran contains a larger portion of sub aleurone starchy endosperm than wheat bran, because the aleurone layer of oats does not separate as cleanly from the endosperm as it does in wheat. The aleurone layer contains typically one cell layer, has a thickness of 50–150 µm (Miller & Fulcher, 2011), and is quite resistant to digestion.

2.1.1.3 Starchy endosperm

The largest part of the oat groat is the endosperm, which may constitute 55-80% of the oat grain by mass. In contrast to the bran, the cells in the endosperm are generally larger and the

cell walls thinner. The largest tissue in oat grains is starchy endosperm which, depending on the variety, may constitute 55–70% of the dehulled oat groat. The endosperm is composed of cells of only one type, each of which consists of starch, protein and lipids (Table 2.1). Starch is the major single component in oat endosperm as well as in whole groats. It occurs as a cluster of several starch granules. The diameter of the aggregates range from 20 to 150 µm, and the size of the individual granules is 2– 15 µm across (Hoover & Vasanthan, 1992; Sowa & White, 1992).

Table 2.1 Oat groats composition (Adapted from Miller & Fulcher, 2011)

Component	Range (%)
Protein	11-20
Fat	5-9
Starch	44-61
Total dietary fiber	7-11
B-glucan	2.2-6.6
Free sugar	0.9-1.3
Moisture	9-14

2.1.1.4 Germ

The third fraction, the oat germ, contains 30-35% of the total oat protein content and 10% of the total lipid content. Germ is a viable structure, capable of metabolic activity, from which a new plant starts to sprout. The germ contains high amounts of protein and lipid but very little starch (Miller & Fulcher, 2011).

2.2 Oats carbohydrate

The largest portion of oat grain is comprised of carbohydrates covering 60% of the total dry mass of the cereal grain (Zhu, 2017) which lies in the common carbohydrate range of other cereals. Table 2.1 indicates the composition of oat groats. Carbohydrate is a broad description of a wide variety of molecules which includes simple sugars, starch (sugar polymers), and non-

starch polysaccharides (Peterson, 1992; Zhu, 2017). Carbohydrates can be grouped by a variety of factors such as solubility, digestibility, starch and non-starch polysaccharides. Literature generally reports two broad component categories: starch and non-starch polysaccharides, which includes soluble sugars as well as fiber (including soluble polysaccharides, insoluble polysaccharides and lignins) (Peterson, 1992).

2.2.1 Starch

Starch is the major part of grains and generally accounts for up to 80 % of the total carbohydrate present, depending on the cereal of interest (R. Welch, 1995). Different studies have shown different starch content and some authors report that starch falls in the range of 60 to 73 % of total carbohydrate present in the oat grain (Ovando-Martínez, Whitney, Reuhs, Doehlert, & Simsek, 2013). It is considered to be the primary energy source in the endosperm, which also plays an important role during germination process of grain (Peterson, 1992).

2.2.1.1 Molecular Composition of Starch

Starch has two key components, amylose and amylopectin as shown in Figure 2-2, and it is known as a storage polysaccharide. (Ellis et al., 1998; Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005).

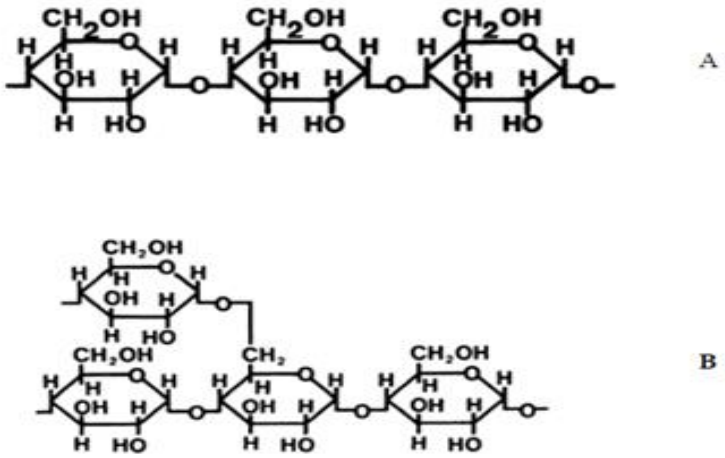


Figure 2-2 The typical structure of (a) amylose and (b) amylopectin

Oat starch can contain about 20-25% amylose, which is a relatively straight chain polymer of D-glucose, consisting α(1→4) glycosidic linkages and less than 1 % branching α(1→6) linkages (Figure 2-2) (Ellis et al., 1998; MacArthur & D'Appolonia, 1979; Zhou, Robards, Glennie-Holmes, & Helliwell, 1998). Amylose is reported to have a molecular mass of 10^5 to 10^6 Da (Van Der Borght et al., 2005). Amylose is essentially a linear molecule and the degree of polymerization of the individual molecules is reported as 939 to 1208 glucose units for oat starch compared to 1500–6300 glucose units in wheat starch (Zhou et al., 1998).

Where amylose is formed from several glucose units arranged in a relatively linear manner, amylopectin is structured with glucose polymers in a branched manner comprising of both straight chain α (1→4) and branched α (1→6) linkages (Figure 2-2). A molecular mass of > 10^8 Da is reported in literature for amylopectin (Van Der Borght et al., 2005) and comprises about 80% of starch. Branching happens to be around every 20 to 25 glucose units, depending on the cereal, and the degree of polymerization of the individual molecules are reported as 104 to 107 glucose units for wheat starch. The degree of polymerisation of the amylopectin fraction of oat starch has been measured as 17 to 204 (weight average) glucose units (Wang & White, 1994b; Zhou et al., 1998). Amylopectin has a lower solubility than amylose in hot water but forms a gel in hot water which is not the case of amylose. The main fractions of oat starch i.e. amylose and amylopectin are presented in the Table 2.2.

Table 2.2 Starch component of oat (Adapted from Tester & Karkalas, 1996)

Starch Fraction	**Mass Fraction (%)**
Total amylose	27.5-29.8
Apparent amylose	19.7-22.0
Lipid	0.66-0.75

The degree of polymerization of the amylose and amylopectin fractions of oat starch has been reported in previous studies as 939 to 1208 (mass average) and 392 to 2920 (apparent) glucose units for amylose and 17 to 204 (weight average) glucose units for amylopectin (Wang & White, 1994b).

Several kinds of protein are found in cereals, some of them are attached to the surface of starch granule (surface proteins), while the others are matrixed within the starch granule (integral proteins) (Ellis et al., 1998). Oat starch contains minor amounts of both protein and lipid but has higher levels of lipids than other cereals (Sowa & White, 1992; Wang & White, 1994a). The lipids are primarily comprised of phospholipid and free fatty acids and these amylose lipid complexes in oat starch are found in varying ranges from 1-3%, which are relatively higher when compared to wheat and maize starches (Hoover & Senanayake, 1996; Sowa & White, 1992; Wang & White, 1994a)

2.2.1.2 Starch Granules

Oat starch is the most abundant component that makes up 55 to 60% of the oat grain and is mostly present as starch granules. Different oat cultivars are reported to exhibit different size and shapes of starch granules (Ellis et al., 1998; Van Der Borght et al., 2005). They can be found in both single granules and agglomerated clusters of granules usually termed "compound-granules" (Tester & Karkalas, 1996). Individual oat starch granules are smaller than the compound granules ranging from 3 µm to 12 µm and 60 µm to 150 µm in size respectively (Zhou et al., 1998). The individual oat starch granules are polyhedral in shape and comparatively smaller in size than other common cereals, such as wheat (Ellis et al., 1998; Hoover & Vasanthan, 1992; Sowa & White, 1992; Tester & Karkalas, 1996).

2.3 Starch Functionality

Investigating the various studies published already, it is found that the discussion over the functionality of oat starch is less when compared to its non-starch components such as the

soluble sugars and the fiber component, in particular the β-glucan (Paton, 1977; Tester & Karkalas, 1996). Like all other cereal starches, oat starch granules are insoluble in cold water, but show some water binding capacity (0.4 g water per 1 g starch). When these starches are dissolved in hot water or boiled into water, they swell in size. The whole process of irreversible solubilization of starch in water to form a paste is known as “gelatinisation” (Ellis et al., 1998). An increase in the solubility of starch is reported with the increasing temperature from 4.1 % to 6.0 % at 85 °C to 33.5 % to 43.3 % at 95 °C (Wang & White, 1994a). Swelling power is also another way to measure the amount of water used into the starch granule which is often used to describe the changes in starch granules during heating and gelatinization.

The gelatinization of oat starch is characterised by different factors such as gelatinization onset temperature, gelatinization peak temperature, and gelatinization conclusion temperature which are reported to vary with cultivar, as does with the swelling power (Ellis et al., 1998; Paton, 1977; Tester & Karkalas, 1996). Literature reports that the gelatinization properties of oat starch differ from other cereal starches with oat starch having a comparatively lower gelatinization temperature (MacArthur & D’Appolonia, 1979; Paton, 1977; Sowa & White, 1992; Wang & White, 1994a). Literature reports that oat starch has an unusually high viscosity on cooling, with the resultant cooled gel being clearer, less firm, more elastic, more adhesive and less susceptible to degradation than cooled starch gels from other cereals such as wheat (Berski et al., 2011; Zhu, 2017).

2.4 Milk proteins

Milk is a complex heterogeneous fluid, and its principle constituents are varied crucial nutrients such as proteins, fat, sugar, minerals and several other minor vitamin components (Farrell et al., 2004). Milk is a rich source of numerous bioactive components such as oligosaccharides, glycolipids, enzymes, growth factors, hormones, vitamins, proteins and

peptides (Hoppe et al., 2008; Onwulata et al., 2001). The structure of milk protein has been studied extensively, and hence milk proteins are well characterised based on their physico-chemical and functional properties (Table 2.3). The proteins present in milkcomprise caseins and whey proteins. Around 80% of the total protein present in milk is constituted by caseins, which form colloidal suspensions called micelles (clusters of casein molecules are associated with calcium and inorganic phosphate). These micelles are the main reason for the white appearance of milk because of their large molecular size (20–200 nm) which scatter (reflect) light and embellish them with white appearance. Around 20 % of total milk protein is whey protein comprised of α-lactalbumin (α-lac), β-lactoglobulin (β-lac), bovine serum albumin (BSA) and other minor proteins (Table 2.3).

Table 2.3 Milk composition (Adapted from Farrell et al., 2004)

Protein/peptide	Concentration (g/kg of solids)	Total Protein (%)	Amino acid residues	Molecular weight (kDa)	Isoelectric focusing point (pI)
Casein					
α-s1-casein	12.0–15.0	30.6	199	23.6	4.9-5.3
α-s2-casein	3.0-4.0	8	209	25.3	4.9-5.3
β-casein	9.0-11.0	30.8	209	24	5.2
K-casein	2.0-4.0	10.1	169	19	5.8
Total-casein	26	79.5			
Whey protein					
α-Lac	0.6-1.7	3.7	123	14	4.4
β-lac	2.0-4.0	9.8	162	18.3	5.4
BSA	0.4	1.2	582	67	5.1
LF	0.02-0.1	0.3	690	77	7.9
LP	0.03	0.1	612	78	9.6
Immunoglobulins	0.7	2.1		150-1000	5.0-8.0
Total whey proteins	6.3	19.3			
Total proteins	32.7	100			

2.4.1 Casein Proteins

Being a good source of essential elements such as amino acids, calcium and inorganic phosphates, caseins play a very important role in providing nutrition to the human body

(Brunner, 1981). These proteins are divided into four major groups: α-s_1, α-s_2, β, and κ-caseins comprising 45, 12, 33 and 10% respectively, of total casein proteins (Farrell et al., 2004; Moughan, 2008). Caseins have a micelle forming structure, the individual characterization of casein proteins is complicated and thus they show communal properties. The major subunit of protein in bovine milk is α-casein, which can be coagulated in the presence of minor levels of calcium. β-casein also shows sensitivity towards calcium and show similar properties (Farrell et al., 2004) while κ-casein remains unaffected by calcium presence. Unlike the casein proteins, κ-casein shows solubility in calcium solution under conditions and also plays a critical role in the stabilization of caseins by forming casein micelles in the presence of calcium.

The number of amino acid and average molecular weight are greater for α-caseins and β-caseins than κ-casein i.e. 200-210 amino acid residues, with an average molecular weight of 24 kDa, and 169 amino acid residues with a molecular weight of 19 kDa respectively. Casein P\proteins are also considered as phospho-proteins because they contain a higher percentage of proline (phosphorus containing amino acid) than other proteins (Farrell et al., 2004).

2.4.2 Whey proteins

Whey proteins are a rich and balanced source of sulphur containing amino acids such as cysteine and methionine, and are known to be highly soluble in aqueous buffers (Farrell et al., 2004). Wheyhas a wide range of applications in food, health, pharmaceutical and cosmetic industries as it contains a mixture of different proteins and peptides and their functionality including gelation, emulsification, chelating agent, antioxidant activity, foaming, thermal stability, solubility, flavor binding and water-binding capacity (Hoppe et al., 2008; Madureira et al., 2007; Onwulata et al., 2001). Whey protein is a group of proteins found in milk and typically known as globular proteins. They are sensitive to heat treatments when compared to caseins and heating above 65-70 °C can denature the protein structure. On the other hand,

they show stability to pH variations and do not coagulate at pH 4.6. Whey proteins are a rich source of essential amino acids and they are found to be very useful in human health (Madureira et al., 2007). Whey protein has all the essential amino acids in higher amount compared to different vegetable proteins sources (Walzem, Dillard, & German, 2002). The major portion of whey protein is occupied by β-lac which is approximately 50–60% of the total whey proteins in bovine milk. The molecular weight of the monomeric form of β-lac is 18.4 kDa with 162 amino acid residues (Billakanti, 2009). β -lactoglobulin is a source of the essential and branched chain amino acids. Various nutraceuticals and food companies might have a wide range of application of β-lac in their products due to gelling and foam stabilizing properties. It can be selectively removed from bovine whey and utilised in the formulation of infant food products (Billakanti, 2009; Eugenia Lucena, Alvarez, Menéndez, Riera, & Alvarez, 2006). Human milk is considered the richest source of α-lac whey protein followed by bovine milk whey. Around 20% of total bovine whey protein is composed of α-lac with a molecular weight of approximately 14 kDa with 123 amino acid residues. Tryptophan and cysteine are the two essential amino acids which are found in higher amount in α-lac whey protein (Madureira et al., 2007).

2.5 Dairy protein-Starch interactions

2.5.1 Casein protein-strach interactions

Starch gelatinisation is a process of swelling of starch molecules in the presence of water with constant heat, and results in the breaking down of starch crystalline structure, allowing for the release of amylose and amylopectin into the system. This leads to increase in viscosity, a number of food formulations involve the pasting of starch in the presence of dairy proteins such as oat porridge and rice, semolina pudding. Casein proteins are commonly used in the form of sodium caseinate or calcium caseinate, acid casein and rennet casein. Lelièvre and Husbands (1989) examined the effect of sodium caseinate on corn and waxy maize starch and

reported that the presence of sodium caseinate increased the viscosity of starch paste. The swelling volume of starch granules increased in the presence of sodium caseinates with a synergistic effect that leads to shear thinning. The same results were reported later by Doublier (1994), who illustrated an increase in viscosity and the reduction of the thixotropic behaviour of starch pastes in a sodium caseinate-starch system of wheat, potato and tapioca, they showed a large drop in solubility associated with the addition of sodium caseinate. The authors concluded that the effect of sodium caseinate on rheological properties was predominently related to both concentration and botanical origin of the starch, this observation was also established by other researchers in further studies (Bertolini, Creamer, Eppink, & Boland, 2005; Kelly, Van Wagenberg, Latham, & Mitchell, 1995). Bertolini et al. (2005) studied the behaviour of cassava, potato, amylomais corn, waxy corn, and wheat starch with different concentration of sodium caseinates. Each starch exhibited different rheological behaviour. However, the addition of sodium caseinate increased the storage modulus (*G'*) in all the starches. Variations in the behaviour were observed at different concentrations of sodium caseinates. Rice and wheat showed a similar trend as their elastic properties increased even at a low concentration of sodium caseinate, however, the viscoelastic properties of cassava starch were more pronounced at higher concentrations, this could be due to a lower natural viscosity of cassava starch at lower concentrations of sodium caseinate. Thus, the botanical origin of starches, and it's clear that potato starch has unique behaviour in comparison with cereal starches. The effect of sodium caseinate on potato and maize starch was studied by Kelly et al. (1995) to explore the behaviour of different starches in the presence of proteins. They reported that even a slight concentration of sodium caseinates (0.01%) resulted in a decrease in starch paste viscosity, while the maximum effect was seen at 1% concentration. An increase in apparent solubility was also observed as the low levels of

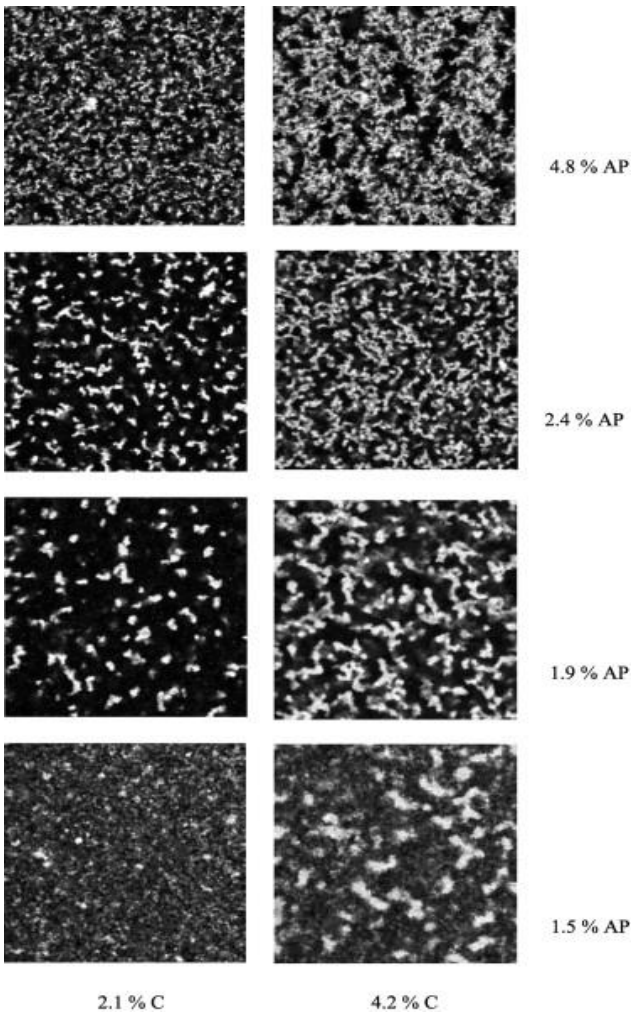


Figure 2-3 CLSM images of milk constituents mixtures containing 2.1 and 4.2% casein (c) respectively and 1.5, 1.9, 2.4 and 4.8% amylopectin (AP); white color=protein rich area; black color=amylopectin rich area. This figure is adapted from de Bont et al. (2002)

sodium caseinate concentration were increased from 0 to 0.06%, this was attributed to a reduced release of starch components from starch granules; hence the increase in solubility can be credited to caseinates. The authors also studied the polyelectrolyte behaviour of potato starch as affected by ions associated with casein. They reported a significant reduction in viscosity due to the addition of small quantities of salt; however dialysed caseinate showed intermediate flow behaviour in comparison with undialysed caseinate and potato starch (1%) viscosity profiles. It was concluded that the reduction in viscosity was due to the unique polyelectrolyte behaviour of potato starch as a result of its high level of phosphate groups and

the large sodium caseinate molecules rather than protein-starch interactions. Bertolini et al. (2005) also reported a decrease in storage modulus (*G'*) in potato starch gels when sodium caseinates were added, this was again attributed to unique polyelectrolyte behaviour of potato starch. In another study by Zaleska, Ring, and Tomasik (2001), they confirmed the interaction of the functional group of potato starch and casein proteins by infrared spectroscopy and electrosynthesis. The band shift was observed in the region of CO-NH group (~1650 cm^{-1}), NH moiety (~1530 cm^{-1}) and OH group bending modes of starch and casein between 1370-1270 (cm^{-1}), which indicated possible interactions between the amino groups of the milk protein and the phosphate groups of the potato starch. de Bont et al. (2002) studied the phase separation behaviour of the mixture of milk permeate (colloidal casein) obtained from the stock solution of skim milk powder (SMP) of 28.6% (w/v) and amylopectin (10% w/v) extracted from potato starch. They observed no phase separation behaviour between the amylopectin (1%) and the casein (2.6%) which exhibited a stable homogeneous matrix, however as the amylopectin concentration was increased to more than 1%, two layers were formed. Higher salt concentration could be responsible for more phase separation between starch and protein. It was reported by Kelly et al. (1995) that phase separation between starch and caseinate was more pronounced at higher levels of salt concentration. It was assumed that high salt concentration encouraged the phase separation between non-polyelectrolyte starch and polyelectrolyte caseinates by hindering the unfavourable entropy change caused by counter-ion concentration at phase separation. Confocal laser scanning microscopy (CLSM) images also supported phase separation, with protein rich areas (white) and amylopectin rich areas (black) as amylopectin concentration was increased (Fig.2-3). The protein network was observed to be more stiff at high levels of either casein or amylopectin and successfully applied vrij's depletion theory. Vrij's depletion theory can be understood

regarding attractive interaction between the colloidal particles. de Bont et al. (2002) also observed an increase in both *G'* and *G''* with time which indicated changes in the microstructure of the matrix. After 3 h, *G'* was found to be greater than the loss modulus (*G''*), which supported a gel like formation instead of paste between the milk protein and amylopectin molecules during phase separation (Fig 2-4).

Noisuwan et al. (2011) examined the adsorption behaviour of sodium caseinates and whey protein isolate (WPI) on normal and waxy starch granules of rice. Centrifugation was used to promote the adsorption of sodium caseinates and adsorption of proteins on to the starch granule surface was confirmed by SDS-PAGE. This study indicated a substantial adsorption of α_s-casein and β-casein on both types of rice starch granules. However, normal rice starch granules showed greater adsorption of casein sub-fractions on to the starch granules in comparison with waxy starch granules (Noisuwan et al., 2011). Such an observation could be due to the surface characteristics of the starch granule, as a normal starch granule has more lipids and proteins than waxy starch and these lipids and proteins could participate in intermolecular bonding with milk proteins via covalent and noncovalent bonding (Seguchi, 2001). At low concentrations of sodium caseinates, a gradual increase of α_s-casein and β-casein adsorption was observed as the concentration of sodium caseinates was increased, a plateau value of 0.35 mg/m^2 and 0.25 mg/m^2 respectively was reached, at 0.25% added sodium caseinates (Noisuwan et al., 2011).

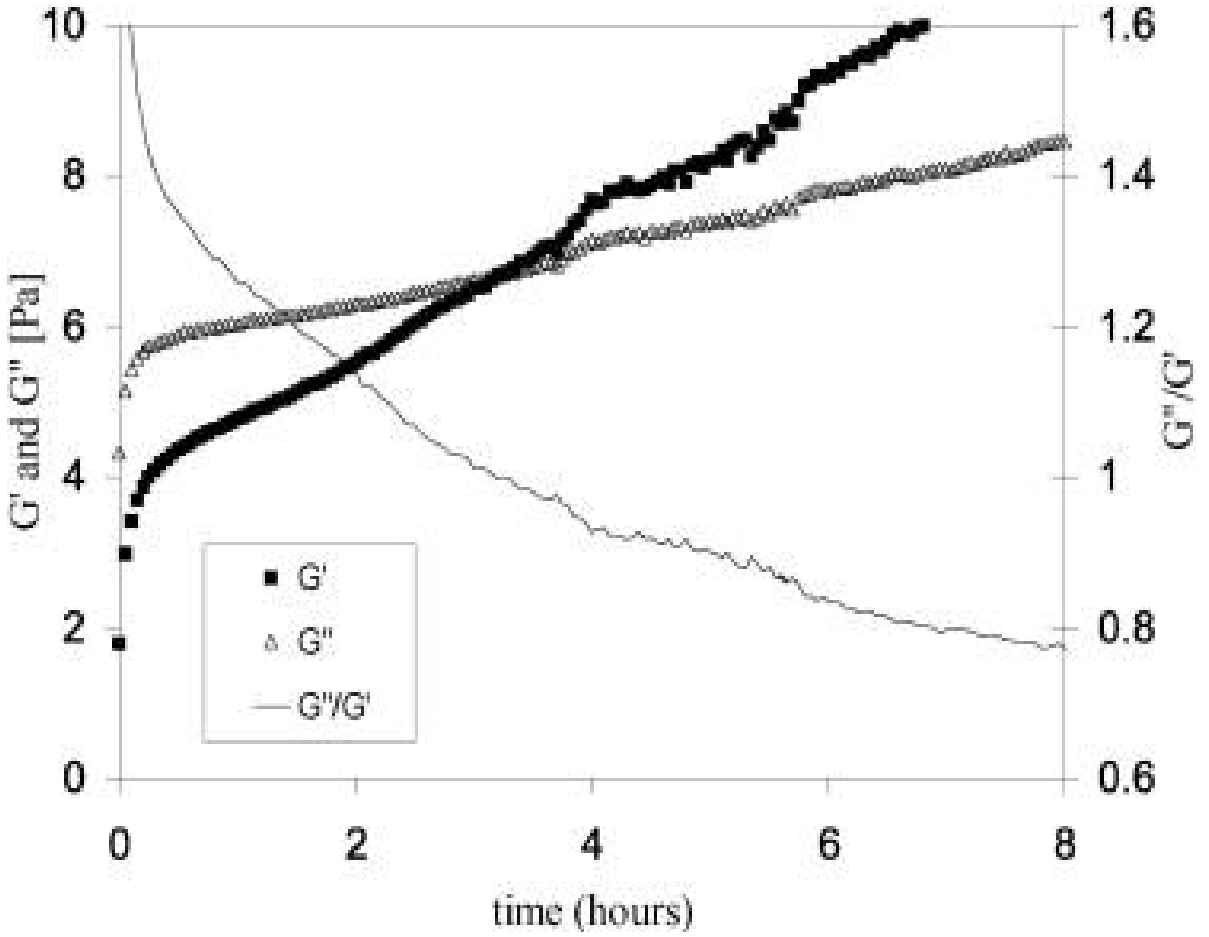


Figure 2-4 Dynamic moduli and tan δ (=G"/G') as a function of time for a mixture containing 2.7% casein and 4.8% amylopectin. This figure is adopted from de Bont et al. (2002).

Noisuwan et al. (2007) explored the effect of sodium caseinate, WPI and SMP on viscosity and the leaching behaviour of normal and waxy rice starch granules. The authors reported that the viscosity onset temperature was increased by the addition of SMP and sodium caseinate to normal rice starch and waxy starch, while WPI did not affect the onset temperaturein both starches. Their studies demonstrated that leaching of amylose was minimal during the onset of swelling and more amylose leached from normal rice starch in comparison with waxy starch. However, leached starch consisted of both amylose and amylopectin biopolymers. The swelling onset temperature of normal rice starch was increased from 56.2 ^{o}C to 58.6 ^{o}C and 60.3 ^{o}C when sodium caseinate and SMP were added, respectively. However, this result was supported by another study of Noisuwan et al. (2008), in which they

explored the effect of milk protein concentrate (MPC), WPI, SMP and sodium caseinate on normal and waxy rice starch. Onset temperature of normal rice starch remianed unaffected by the addition of MPC, WPI and sodium caseinate, however, SMP addition increased the onset temperature in both starches, possibly due to the presence of lactose and salts. Bertolini et al. (2005) also suggested that sodium caseinates increased onset temperature, gelatinisation temperature and end temperature of the gelatinisation process in all the starches. This was explained by the lack of enough water to starch during gelatinisation, which was more evident in high amylose content starches. Using light microscopy studies these authors also observed a more pronounced homogeneous matrix in starch paste due to the addition of sodium caseinates. Goel, Singhal, and Kulkarni (1999) studied the interactions between corn starch and casein and casein hydrolysate , in which casein and casein hydrolysate used in substituting 11.1 - 44.4 % (w/w) of starch. Casein protein was hydrolysed by enzymic and acid hydrolysis method to yield various level of α-amino nitrogen. A decrease in gelatinisation temperature and an increase in cold viscosity were observed as the casein concentration (11.1 - 44.4 % w/w) increased, however, casein hydrolysate had no influence on these attributes. A decrease in apparent viscosity was observed in all the mixtures which suggested further shear thinning of starch pastes over time.

Noisuwan et al. (2009) reported a slight increase in storage modulus (*G'*) with the increase of oscillatory frequencies in both normal and waxy rice starches, but they experienced an overall weak gel structure in small deformation rheological measurements. Large deformation frequency-sweep studies demonstrated a transformation from elastic to the viscous nature as *G''* became greater than *G'* at high strain values (*G''*> *G'* at >600% strain). An increase in viscosity at a low shear rate (10 s^{-1}) was also reported by Bertolini et al. (2005), but the gels lost their structural network as the shear rate was increased, which suggested a brittle gel

network.

Kett et al. (2013) examined the effect of gelatinisation of 5 % (w/w) waxy maize starch with 5 % (w/w) α-lactalbumin (α-lac), β-lactoglobulin (β-lac) and α-caseinate or β-caseinate by CLSM imaging. Both caseinates appeared to reinforce the starch granule structure, however,

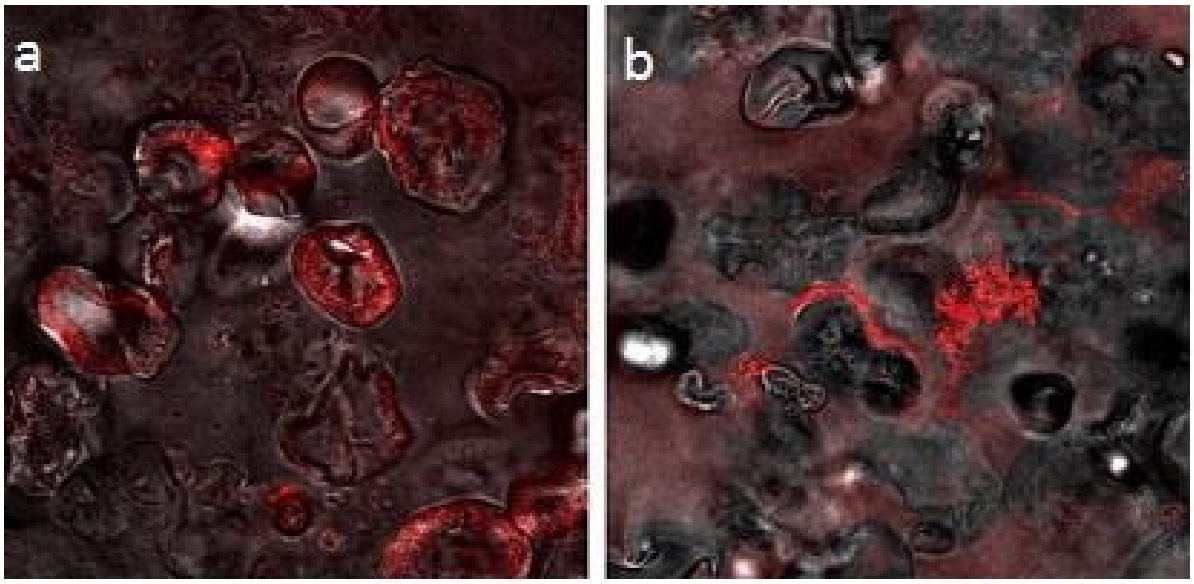


Figure 2-5 CLSM images of 5 % (w/w) waxy maize starch (pH 7.5) after gelatinisation at 70 °C with 5 % (w/w) α-caseinate (a) and 5 % (w/w) β-caseinate (b). Red color indicates protein (scale bar=50µm). This image is adapted from Kett et al. (2013).

the whey protein subfractions (α-lac and β-lac) were not observed to follow this trend in CLSM micrographs. The authors observed a prominent presence of protein in continuous phase at 60 °C (below the pasting temperature) and adsorption of protein on to starch granules. A similar adsorption effect was reported by Noisuwan et al. (2011) at ambient temperature, adsorption of β-lac was higher than α-lac and increased as the concentration of WPI was increased. However, at 70 °C, α-caseinate penetrated into swollen starch granules (Fig 2-5a), no such penetration was observed for β-caseinate. CLSM micrographs showed adsorption of large aggregates of β-caseinate on the swollen starch granules surface and in the continuous phase (Fig. 2-5). This phenomenon was explained by self association of β-caseinate molecules

due to their shape and the presence of hydrophilic and hydrophobic end groups giving it the capability to form micelles in the same way as detergents, whereas self association is not only limited but also reversible for α-caseinate. The mechanism behind the adsorption of casein micelles could be attributed to lipid protein layer of starch granules.

2.5.2 Whey protein-starch interactions

Whey-starch interactions are important to understand because of their global use in foods, and their ability to form gels under certain conditions. Whey proteins have a tendency to form gels and as such they could cause greater interference than caseins in starch gelatinisation or in a system where thermal processing is used. Whey proteins consisted of heterogeneous mixture of β-lactoglobulins and α-lactalbumins, which make up almost 80% of total whey protein present in milk. However, the functional properties of whey proteins are attributed to β-lactoglobulins. Therefore, this section discusses to WPC, WPI and β-lactoglobulin-starch systems interactions.

Olsson et al. (2000) studied the influence of non-gelling potato amylopectins on β-lactoglobulin gels by light microscopy and rheological methods. The authors reported that high viscosity potato amylopectin had a shear thinning effect on β-lactoglobulin (6 wt%), while low viscosity amylopectins had a more Newtonian behaviour. Aggregation of β-lactoglobulins was influenced by the concentration and type of potato amylopectins, a higher concentration of amylopectins produced more prominent and larger pores between β-lactoglobulin strands aggregates. Quiroga and Bergenståhl (2008) also observed a similar effect of shear thinning behaviour of β-lactoglobulin and waxy maize starch samples at high amylopectin concentrations, as apparent viscosity decreased with increased shear rate. In another study by Olsson et al. (2002), these interactions were further investigated using CLSM and transmission electron microscopy (TEM). The authors found that the increased concentrations

of amylopectin resulted in a more closely packed structure of β-lactoglobulin aggregates. The cluster size, pore size and distance between cluster strands had an effect on storage modulus as well as stress at fracture. Heated mixtures of WPI and cross linked maize starch (CWM) showed the same phenomenon, where (*G'*) showed a slight increase with time. A decrease in the phase angle (tan δ= *G''/ G'*) supported the gradual accumulation of β-lactoglobulin aggregates structure (Ravindra et al., 2004). Fitzsimons et al. (2008) explored WPI (2 wt% and 10 wt%) and phosphate crosslinked waxy maize starch (PCS-10 wt%) heated co-gels by differential scanning calorimetry (DSC). DSC heating traces showed two overlapping endothermic transition peaks for WPI and starch mixtures at both concentrations of WPI, the resulting peaks represented a typical gelatinisation endotherm peak (63 ^{o}C) and β-lactoglobulin endotherm peak (74 ^{o}C) (Fig. 2-6). Studies with DSC revealed that a mixture of WPI and starch enhanced thermal stability of mixture, but did not support a direct association of both biopolymers. The microscopic observations of Vu Dang et al. (2009) also indicated swollen starch granule as a dispersed phase and the continuous WPI phase was found to be heterogeneous and observed large protein aggregates near the dipersed phase of starch granules in WPI/cross-linked waxy maize starch mixtures. The authors suggested that these protein aggregates may result in a grainy appearance of food products.

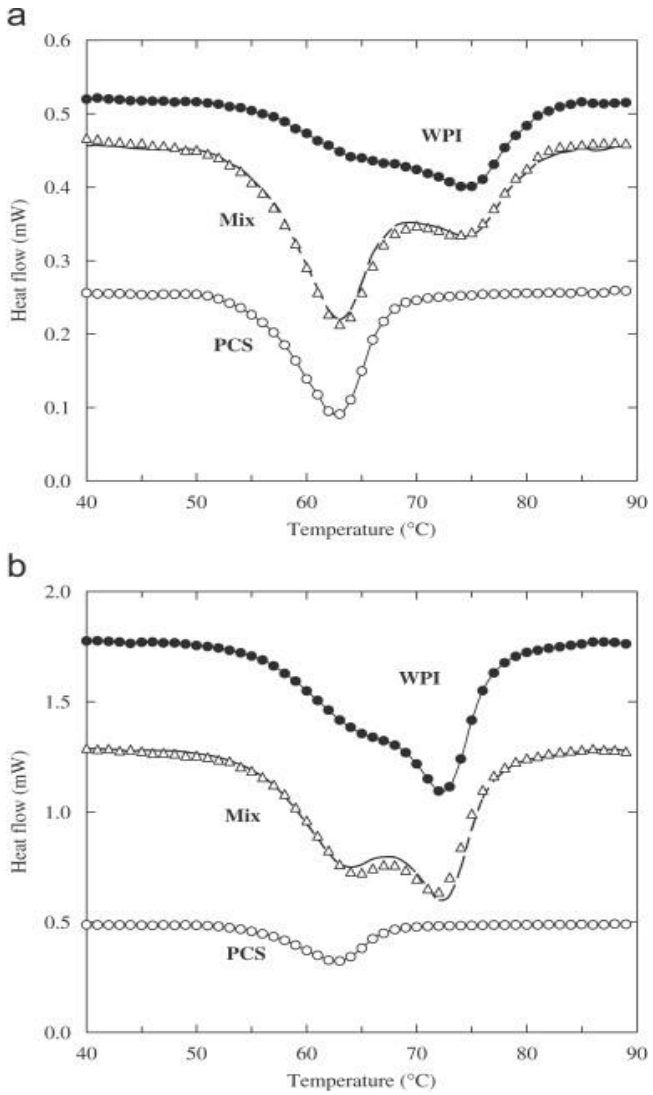


Figure 2-6 DSC heating curves for PCS alone (○), WPI alone (●) and a mixture of both (Δ), recorded at polymer concentrations of (a) 2 wt% PCS; 2 wt% WPI and (b) 2 wt% PCS; 10 wt% WPI. This figure is adapted from Fitzsimons et al. (2008).

Herceg et al. (2007) explored the effect of whey protein concentrate (WPC), WPI and β-lactoglobulin with mixed carbohydrates, including starch, on foam development, stability and emulsifying properties. Starch impacted negatively on foam expansion and stability as well as on the emulsifying properties of WPI, WPC and β-lactoglobulin.

Recently, Matignon et al. (2014) studied a starch/carrageenan/milk mixture using multiple staining techniques and CLSM. They found no physical interactions between milk proteins and starch granules during pasting of the carrageenan/milk mixture. However, a higher breakdown

of starch granules was observed in milk solutions in comparison to pasting in water or in a carageenan solution. The authors presented no justification for this result, though it could be due to presence of salts, protein and sugars present in milk. Matignon et al. (2015) also investigated skim milk components and thermo-mechnical treatment on swelling behaviour and rheological properties of starch. A short thermo-mechanical treatment did not affect swelling of the starch granules. However, prolonged heat treatment led to bigger starch granules in both laboratory and pilot scale experiments. Yang, Ashton, and Kasapis (2015) investigated medium molecular weight chitosan, WPI and native wheat starch in composite matrices, and found that at a low concentration of starch. The gel firmness was reduced in the tertiary composite matrices. Results from the DSC indicated two peaks, the first peak was attributed to starch gelatinisation while the second peak observed was attributed to the protein denaturation. However, this result showed no direct chemical or elerctrostatic interaction between the starch and chitosan/WPI system.

Even in more complex food systems, whey protein and starch have a prominent effect on the product's physical and chemical properties. Incorporation of WPC and potato starch in water chestnut flour (WCF) based cookies were studied by Sarabhai and Prabhasankar (2015). The addition of WPC decreased peak, final and setback viscosity in WCF during pasting. This phenomenon is due to the dilution of starch caused by WPC and possible interaction of whey proteins with leached amylose and amylopectin through non-covalent bonding. The reason behind the decrease in final viscosity could be attributed to lower recrystallization of amylose molecules during cooling. Whey proteins may have acted as a barrier in the process of reordering of amylose molecules by restricting hydrogen bonding between starch molecules. The addition of WPC and potato starch increased the storage modulus, suggesting a stronger and more elastic gluten free cookie dough (Sarabhai & Prabhasankar, 2015). Similar findings

were reported by Sopade et al. (2006) in a wheat starch milk protein system, where addition of WPC and WPI decreased peak, trough and final viscosities during thermomechanical treatment in a rapid visco analyser (RVA).

2.6 Possible Dairy protein-Starch Interactions Hypotheses

Despite the significant studies done in the starch milk proteins systems, no clear trend of these interactions has been experienced. It is well understood that these complex systems depend upon many factors including starch concentration, protein type, protein concentration and thermomechanical process. It can be hypothesised from the reviewed studies that three types of interaction can occur in a starch milk protein system during the thermo-mechanical process.

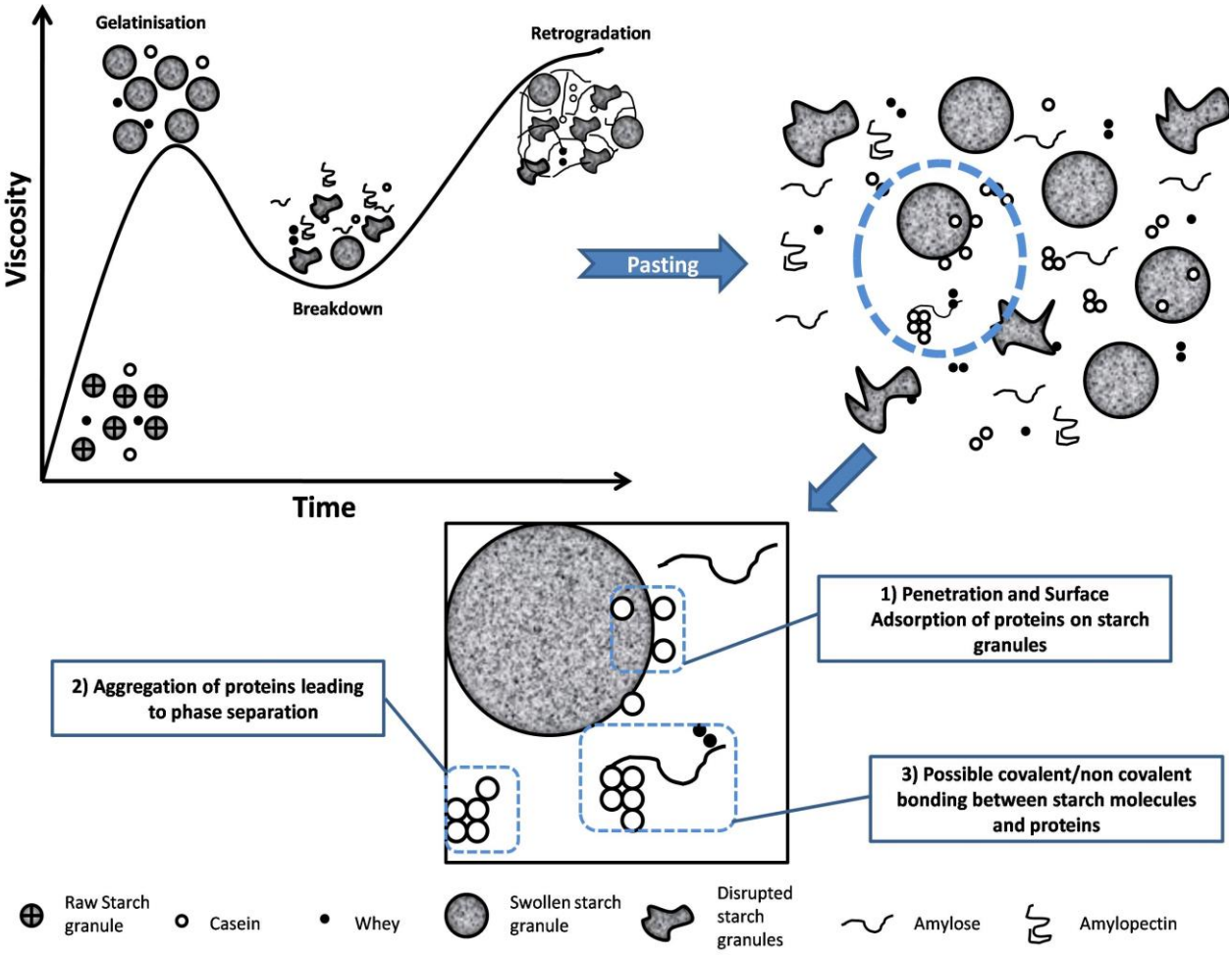


Figure 2-7 Graphical representation of starch/milk proteins interactions during continuous shear heating

(1) penetration and adsorption of proteins on the surface of starch granules (2) protein aggregation in the continuous phase (3) covalent and non covalent bonding between starch molecules and proteins (Fig. 2-7).

As reported by Doublier (1994), the starch protein matrix consists of a dispersed phase and a continuous phase, which is made up of starch granules and protein and amylose/amylopectin respectively. The proteins and starch molecules present in the continuous phase affects starch granule swelling behaviour during pasting. As the pasting continues, swollen starch granules rupture and amylose and amylopectin leach out to form a continuous matrix. Proteins contain many hydrophilic groups such as amide, hydroxyl, carboxyl and thiol in the alkyl side chains, which are capable of forming links with starch molecules (Goel et al., 1999). The study conducted by Yang et al. (2004) also supported non covalent hydrophobic bonding between wheat starch and WPI cooled gels. In addition, whey proteins can interact with exposed branching of amylose and amylopectins side chains by non covalent bonding, it could also act as inactive fillers hindering hydrogen bonding between starch molecules (Sarabhai & Prabhasankar, 2015; Sopade et al., 2006).

Another interaction hypotheses suggests aggregation behaviour of proteins (Fig. 2-7), which is more profound in caseins in comparison to whey proteins and normally leads to phase separation phenomenon. Caseins form large aggregates of proteins around starch granule surface specially β-caseinates, which is due to their micelle forming capacity (de Bont et al., 2002; Olsson et al., 2000; Olsson et al., 2002; Ravindra et al., 2004; Vu Dang et al., 2009). The hypotheses of penetration and physical adsorption of milk proteins on starch granules was postulated by Kett et al. (2013) and Noisuwan et al. (2011). However, these studies showed only the penetration of α-caseinate and the adsorption of casein proteins and their subfractions on starch granule surface. This could be due to bonding between endogenous

lipids and endogenous proteins of starch present on its surface and exogenous dairy protein. Similar studies on wheat starch have also concluded that endogenous proteins present on surface of starch granule mediated adsorption of exogenous proteins (Ryan & Brewer, 2005a, 2005b). The possible hypotheses of intereactions between starch and milk proteins are summerised in Figure 2-7. Despite the recent studies in this area, there has been not enough concrete result which give insight about these interactions during thermal and non-thermal processing. So, to understand the true underlying mechanism, there is a enormous need for understanding the clear phenomenon behind these interactions.

Thus, it can be concluded from all the studies that a certain interaction between proteins and starch molecules occurs which further can lead to product texture and its behavior. However, it depends upon many factors such as concentration, pH and time. Casein protein behaves differently in a starch system compared to whey protein. Both the proteins are responsible for enhancing the gel strength of starch pastes in a certain concentration, while no clear trend in the apparent viscosity of the casein starch mixture has been shown in the literature. However, these proteins can also lead to weak the gel structure by acting as an inert filler or by hindering the reordering of starch molecules during cooling. There is compelling evidence of the effect of sugars and ions such as lactose, phosphate or other charged molecules on the rheological characteristics of a starch-protein system. Caseins have shown a tendency to form large aggregates and have exhibited physical adsorption to the starch granule surface, even α-caseinate can penetrate starch granules. Whey proteins also can form aggregates and be present in a continuous phase or dispersed phase depending upon the processing conditions as they have good gelling properties. It can be well understood that milk proteins affect pasting properties of starch at micro and macromolecular level.

Chapter 3

3 Material and Methods

3.1 Materials

Commercial Harraway's organic oat flour (protein 13.5%, fat 5%, ash 1.65%, moisture 11.5%, dietary fibre 9.2%, total carbohydrate 56.2%) was procured from Piko Wholefoods Organic Supermarket, Christchurch, New Zealand. Whey protein concentrate (WPC) and whey lactalbumin (WLAC) was provided by Oceania Dairy Ltd., New Zealand. WLAC consisted of 30.3% of α-lactalbumin and 18.1% of β-lactoglobulin of total protein. Pams (Auckland, New Zealand) skim milk powder (SMP) was obtained locally. Reactiv pure WPI and CaCN is purchased form online suplement shop at URL https://www.reactivsupplements.co.nz (New Zealand). The chemical compositions of the milk protein ingredients used are reported in Table 3.1 .

Table 3.1 Chemical composition of dairy protein ingredients

Ingredients	WPC[1]	WLAC[2]	SMP[3]	WPI[4]	CaCN[5]
Moisture (%)	3.1 ± 0.3	5 ± 0.5	4.3 ± 0.2	4.7±0.1	4±0.2
Fat (%)	7.2 ± 0.1	10.5 ± 0.5	0.8 ± 0.1	0.3±0.1	1.5±0.4
Protein (%)	77.5 ± 0.1	79.2 ± 0.2	32.6 ± 0.2	93.9±0.3	93.5±0.2
Ash (%)	3.1 ± 0.05	2.9 ± 0.02	8.5 ± 0.03	1.5±0.02	4.5±0.01

Values expressed are means ± standard deviation (n=3).

[1] WPC-Whey protein concentrate, [2] WLAC-Whey lactalbumin, [3] SMP-Skim milk powder, [4] WPI-Whey protein isolate, [5] CaCN- Calcium caseinate

3.2 Methods

3.2.1 Starch extraction

Oat starch was extracted from oat flour using the methods of Paton (1977) and Liu et al. (2014) with slight modifications (Figure 3-1). Dry flour (100 g) was first sieved through a 425

µm screen mesh before starch extraction and a 53 µm sieve was used for removal of coarse bran from starch milk in later stages of extraction. Flour was slurried in 1L of water and the pH adjusted to 10.00 with sodium carbonate. Slurry was screened through a mesh for removal of large bran granules. The slurry was stirred for 30 min at 45 ^{0}C to extract protein and nonstarch carbohydrates. After centrifuging at 3,000 g for 1h the supernatant was discarded, the residue re-suspended in water (pH 10.00) to 1L and extracted a second time. The twice extracted residue was taken up in 500 ml distilled water and the starch milk centrifuged at 1500 g for 30 min. A fine bran layer settled above the sedimented starch and this was carefully removed with a spatula. The starch was suspended in water, neutralized with 1-M HCl, centrifuged at 1,500 g and re-washed as before. Final extracted starch was scraped from tubes by spatula and transferred to petri dishes and was kept in oven at 35 °C overnight for drying.

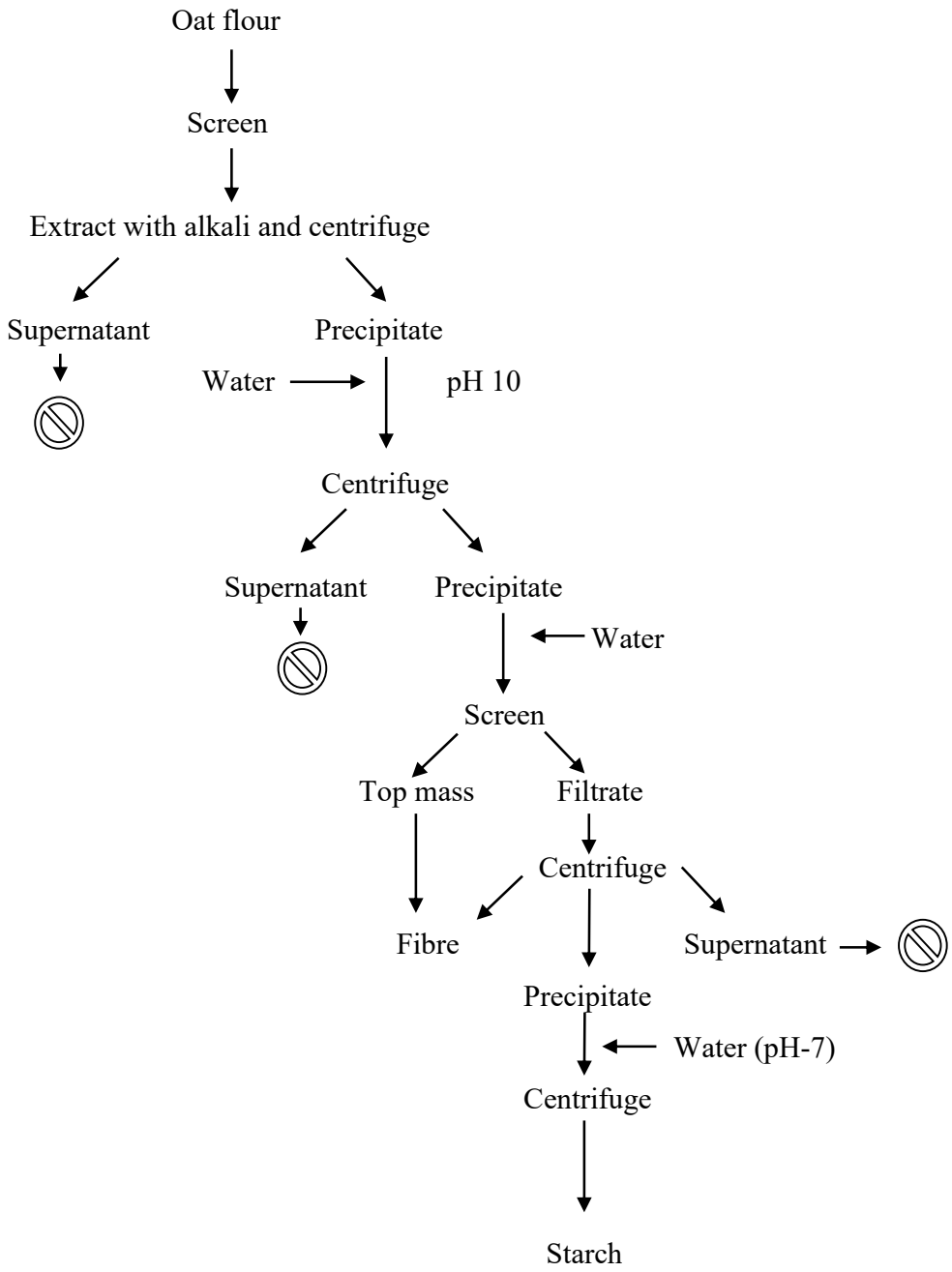


Figure 1. Scheme for starch extraction

Figure 3-1 Schematic representation of oat starch extraction form oat flour

3.2.2 Physicochemical analysis of oat starch and milk protein ingredients

3.2.2.1 Moisture content

Moisture content of the samples were determined by an oven drying method (105 ± 2 °C overnight) described by AACC International Approved Method 44-16.01 (AACC, 2000). The dish was placed in the desiccator for 1 h to allow it to cool to room temperature before reweighing.

$$Moisture\ (\%) = \frac{Loss\ of\ weight}{Sample\ weight} \times 100$$

3.2.2.2 Ash content

The ash content was calculated according to the (AACC, 2000) official method. The crucible containing the sample was incinerated in muffle furnace at 525 °C for 8 h. After that, the crucible was placed in desiccator to cool down and weighed to nearest 0.1 mg.

3.2.2.3 Protein analysis

The protein content was determined using the Element analyser (Vario MAX CN Hanau, Germany) by the Dumas method (Shea & Watts, 1939). Nitrogen conversion factor of 5.83 and 6.38 was used for oat starch and milk protein ingredients, respectively.

3.2.2.4 Fat content

Crude fat content was analyzed by the BUCHI Soxhlet Extraction Unit (E-816HE) (Patil, Brennan, Mason, & Brennan, 2016).

3.2.2.5 Total starch determination

The total starch determination of the oat starch was carried out with the Megazyme Total Starch analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland) following AACCI Approved Method 76-13. 01 (AACC, 2000). Samples were milled and added into glass test tubes. Then, 0.2 mL aqueous ethanol (80 % v/v) was added to the tubes, and test tubes were stirred vigorously. Meanwhile, 3 mL thermostable α-amylase was added and incubated in a boiling water bath for 12 min with stirring after 4, 8 and 12 min. After that, the samples were placed in a bath at 50 °C for 30min and added 0.1 mL amyloglucosidase. The samples were transferred quantitatively to a 100 mL volumetric flask and centrifuged at 3000 g for 10 min. Subsequently, 0.1 mL duplicate aliquots of the dilute solution were added to glass test tubes and each tube was added 3.0 mL GOPOD reagent, then incubated in a bath at 50 °C for 20

min. At last, the absorbance of each sample was determined by spectrophotometer at 510 nm.

3.2.3 Pasting properties

Rapid Visco Analyzer (RVA TecMaster, Perten Instruments, Australia) was used to measure the apparent viscosity of samples as a function of temperature. Starch sample (3 g), with or without (control sample) added milk components, was weighed in the canister and distilled water was added to flour to obtain 28 g sample weight. A programmed heating and cooling cycle standard profile 1 was used where the samples were held at 50 °C and heated to 95 °C at 6 °C /min, a holding phase at 95 °C for 1.5 min, a cooling step from 95 to 50 °C at 6 °C /min, and a holding phase at 50 °C for 2 min. The peak viscosity, trough viscosity, breakdown, final viscosity, setback and pasting temperature were recorded. Derived parameters such as stability ratio (SR), setback ratio and relative breakdown were also calculated from the direct parameters. Stability ratio was the ratio of trough viscosity to peak viscosity, setback ratio was the ratio of final viscosity to trough viscosity and relative breakdown was the ratio of breakdown viscosity to setback viscosity.

3.2.4 Water binding capacity (WBC)

The water binding capacity of different oat starch and milk constituents mixtures were determined by the method described by Sandhu and Singh (2007). A suspension of 1 g of oat starch with milk constituents in 15 ml of water. The suspension was stirred for 1 h and then centrifuged at 3000 g for 10 min. Tubes were inverted for 10 min for complete draining of water and then the wet weight of the mixtures was taken and WBC calculated as the percentage of water absorbed by the mixtures at 25 °C.

3.2.5 Swelling power and solubility index

Starch-milk components dispersions (1%) were heated at 95 °C for 30 min to determine swelling power (SP) and solubility (SOL) following the method of Šubarić et al. (2011). Starch dispersions (1%) were heated at 95 °C for 30 min in a temperature-controlled water bath. After the heating, the samples were centrifuged at 4000 rpm for 30 min. The precipitated paste was separated from the supernatant and weighed. The supernatant was dried at 105°C for 16 h and weighed. The SOL was calculated as the percentage of dry mass of the supernatant to the dry mass of the whole starch sample, while SP was the ratio of the weight of pellet to their dry mass (g/g).

3.2.6 Hydration properties (Water absorption index and Water solubility index)

Water absorption index (WAI) and water solubility index (WSI) were measured as hydration properties by the method of Anderson, (1982) and Choi et al. (2012) with slight modifications. Oat flour (3.0 g) was dispersed in 30 ml distilled water and heated in a water bath at 75 °C for 30 min. After centrifuging the dispersion at 3000×*g* for 15 min, the supernatant was collected in pre-weighed dry aluminum dishes, and the residues were drained off by allowing the sample tube to stand inverted for 10 min. The collected supernatant was dried in a hot air oven at 105°C overnight for WSI measurement. The WAI was calculated as

$$\mathrm{WAI} = \frac{m'}{M}$$

m' = the mass of the wet pellet after centrifugation; M= the dry mass of the pellet.

The WSI was calculated as

$$\mathrm{WSI} = \frac{m''}{m} \times 100\%$$

m'' = dry mass dissolved in the supernatant; m = mass of flour sample.

3.2.7 Freeze-thaw stability

Freeze-thaw properties of oat starch/milk protein ingredients mixture were measured by the method of Hoover & Senanayake (1996) with slight modifications. The oat starch gels (5% w/w) were prepared with different milk constituents in the RVA. Gels were equally distributed into 15 ml tubes (4 g each) in triplicates. Tubes were stored at -20 °C for 21 h and thawed for 3h in hot water bath at 30 °C. This freeze-thaw cycle was given for 5 days and then tubes were centrifuged at 1000 g for 20 min and syneresis was noted after 1st, 3rd and 5th cycle, which is percentage of expelled water ratio to initial weight.

3.2.8 Colour

Colour of starch gels was measured by a Minolta CR-210 Chroma Meter (Minolta Camera Co. Ltd, Osaka, Japan) in terms of L*a*b*. The gels were placed in a 25 mm diameter test tube, the colour was measured three times per sample, turning the test tube 120° during measurements. Results were given as lightness (L*), redness (+a*) and yellowness (+b*). The total colour difference (ΔE) between the control sample and each of the starch gel containing milk constituents was calculated as follows:

$$\Delta \mathbf{E} = \sqrt{[(\Delta \mathbf{L}*)^2 + (\Delta \mathbf{a}*)^2 + (\Delta \mathbf{b}*)^2]}$$

Where ΔL*, Δa* and Δb* are the difference between the two samples in L*, a* and b*, respectively. The values used to determine obvious visual colour difference between samples according to Bodart et al., (2008).

3.2.9 Flow Characteristics

The rheological properties of starch were measured to characterize flow behaviour using a concentric cylinder viscometer (Lamy RM-100, DIN 33 system) which had an inner co-axial cylinder rotating in a stationary outer cylinder. Oat starch paste (5% w/w) with 5% and 10% substitution with milk components (WPC, WLAC and SMP) was prepared in a 600 ml beaker by heating in boiling water bath for 15 min. The samples were stored over night at room

temperature before conducting the rheological tests at 25 °C. The viscometer temperature was thermostatically controlled with a pump powered water circulator (Julabo, TW8). The apparent viscosity (η) of oat flour pastes were recorded over a shear rate varied between 1.29 to 516 (1/s). To predict the flow curves (viscosity; versus shear rate) the Power Law equation was used:

$$\eta = K(\dot{\gamma})^{n-1}$$

3.2.10 Microscopy

The granule morphology of native oat starch was examined by a scanning electron microscope (SEM) (JEOL, JSM-5410LV, Tokyo, Japan). Oat starch was coated with gold in an ion sputter coater (JEOL, JFC-1600, Tokyo, Japan) and the morphology was observed at 800X and 10000X magnification with an accelerating voltage of 5 kV. The effect of thermal processing on starch granule size was determined by measuring the length across the longest axis of 25 granules from a 1% starch suspension of oat starch-milk components mixtures by Nikon-H550S (Nikon Corp., Tokyo, Japan) light microscope using 40X objective lens. A small drop of starch suspension was then put on glass slides and covered with a cover slip and examined under light microscope fitted with a CCD camera (Nikon Digital Sight DS-U3) with a 20X and 40X objective. Live optical images were freezed and processed by NIS Element BR 3.2 (Nikon Corp.) imaging software for granule size measurement.

3.2.11 Syneresis

Oat flour paste (5%, w/w dry basis, total weight 28 g) was prepared using a Rapid Visco Analyzer (Newport Scientific Ltd., Sydney, Australia). The resulting gel was allowed to cool at room temperature for 15 min, and the gel (5 g) was transferred to a 25 ml centrifugal tube in triplicates. The tube was stored at 4°C for 21 h followed by thawing at 30°C for 3 h in a water bath incubator. The tubes were centrifuged at 1000 × g for 20 min, released free water was

carefully discarded, and the tube was drained on tissue paper for 10 min. Syneresis was expressed as

$$\text{Syneresis} = \frac{w}{p} \times 100\%$$

w = mass of water expelled from the pellet; p = mass of the initial paste.

3.2.12 Large deformation viscoelastic textural properties

3.2.12.1 Penetration textural test

Penetration tests were conducted according the method followed by Kumar et al. (2018). The samples were prepared as described in section 3.2.3 and flour paste was transferred to 50 ml plastic vials and stored for 24 h at 4 °C. The paste texture was determined using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd, Surrey, UK) equipped with a Texture Expert software and a 5 kg load was used for measurement. The samples were subjected to compression at a constant crosshead speed of 1 mm/s to a distance of 10 mm with a cylindrical plunger (p/5) for single compression cycle at room temperature. Hardness was defined as the peak force observed during the compression cycle. The distance of penetration at peak force related to the brittleness of the gel. The negative force produced by removal of the probe was defined as adhesiveness, the gradient was measured at the initial linear region of the curve and recorded as 'Elasticity' (Fig.3-2.a).

3.2.12.2 Back extrusion mechanical test

Samples were heated in a boiling water bath with periodic stirring for 15 min and then allowed to cool to ambient temperature (25 °C). The back extrusion textural analysis was conducted at 25 °C as described by Angioloni & Collar, (2009) using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd, Surrey, UK). A back-extrusion cell (A/BE Back Extrusion Rig) was used and a compression disc with an extension bar moved at a speed of 1 mm/s, for 25 mm and pulled back with a speed of 1 mm/s. Three parameters of extrusion were recorded, negative peak

force of the back extrusion (cohesiveness) as well as positive and negative areas of the extrusion. The area under the force-time curve (AUC, in N.s) of positive and negative areas defined consistency and index of viscosity respectively. Higher value of consistency indicated thicker consistency of the sample. The work done indicated by the negative region of the graph, is the result of paste resistance to flow of the disc giving an indication of viscosity. The maximum negative force is defined as cohesiveness of the sample, so that the more negative the value the more 'cohesive' is the sample (Fig 3-2.b).

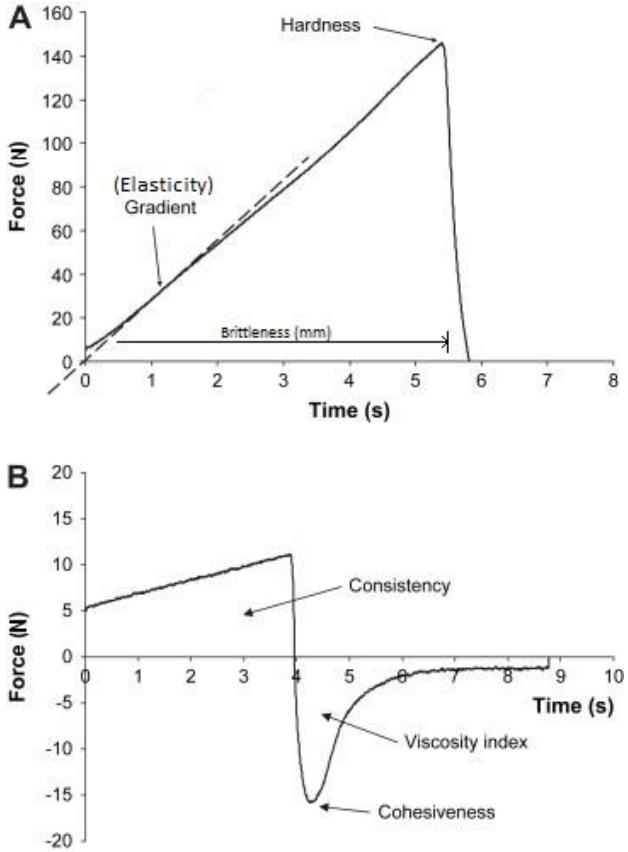


Figure 3-2 Large deformation typical curves (a) penetration and (b) back extrusion (Adapted from Angioloni & Collar, 2009)

3.2.13 Rheometer pasting properties

Pasting properties of starch were studied by using a Rheometer (MCR 52, Anton Paar, Austria) by using a starch cell geometry. Viscosity profiles of starch were recorded using starch suspensions alone and with milk proteins (10%, w/w). Initially the suspension was equilibrated

at 50 °C for 1 min and then heated from 50 to 95 °C at 6 °C /min, then held it at 95 °C for 5 min, followed by cooling to 50 °C at the rate of 6 °C /min and finally held it at 50 °C for 2 min. A constant stirrer speed (160 rpm) was kept throughout the experiment for all the samples, except initially when the speed was 960 rpm for 10 s in order to disperse the sample uniformly.

3.2.14 Differential scanning calorimetry (DSC) analysis

The thermal properties of the native and irradiated starch were investigated by using Differential Scanning Calorimetry (DSC 200, NETZSCH, Germany). Starch samples (3 mg) with WPI/CaCN were weighed in an aluminium pan and deionized water was added to obtain 70% moisture content. The pan was then sealed and kept for 2 h, to obtain equilibrium and proper moisture migration within the sample and then heated from 25 to 130 °C at the rate of 1 °C /min. The instrument was calibrated using indium and an empty aluminium pan was used as a reference. Onset temperature (To), peak temperature (Tp), conclusion temperature (Tc) and enthalpy of gelatinization (ΔH) were determined.

3.2.15 X-ray diffraction (XRD) analysis

The XRD patterns of the dried starch samples with milk proteins were measured by Empyrean PAN analytical diffractometer, equipped with pixel detector employing Cu Ka, running at 45 kV and 40 mA. The samples were analysed between 2 Ø = 10–60° with a step interval of 0.02 and scan rate of 2°/min. Relative crystallinity (RC) was calculated by the ratio of the crystalline area to the total diffractogram area (Nara & Komiya, 1983) (Figure 3-3).

$$RC(\%) = \frac{A_c}{(A_c + A_a)} \times 100$$

Where A_c = crystalline area, A_a = amorphous area and $A_c + A_a$ = total area of XRD diffractogram

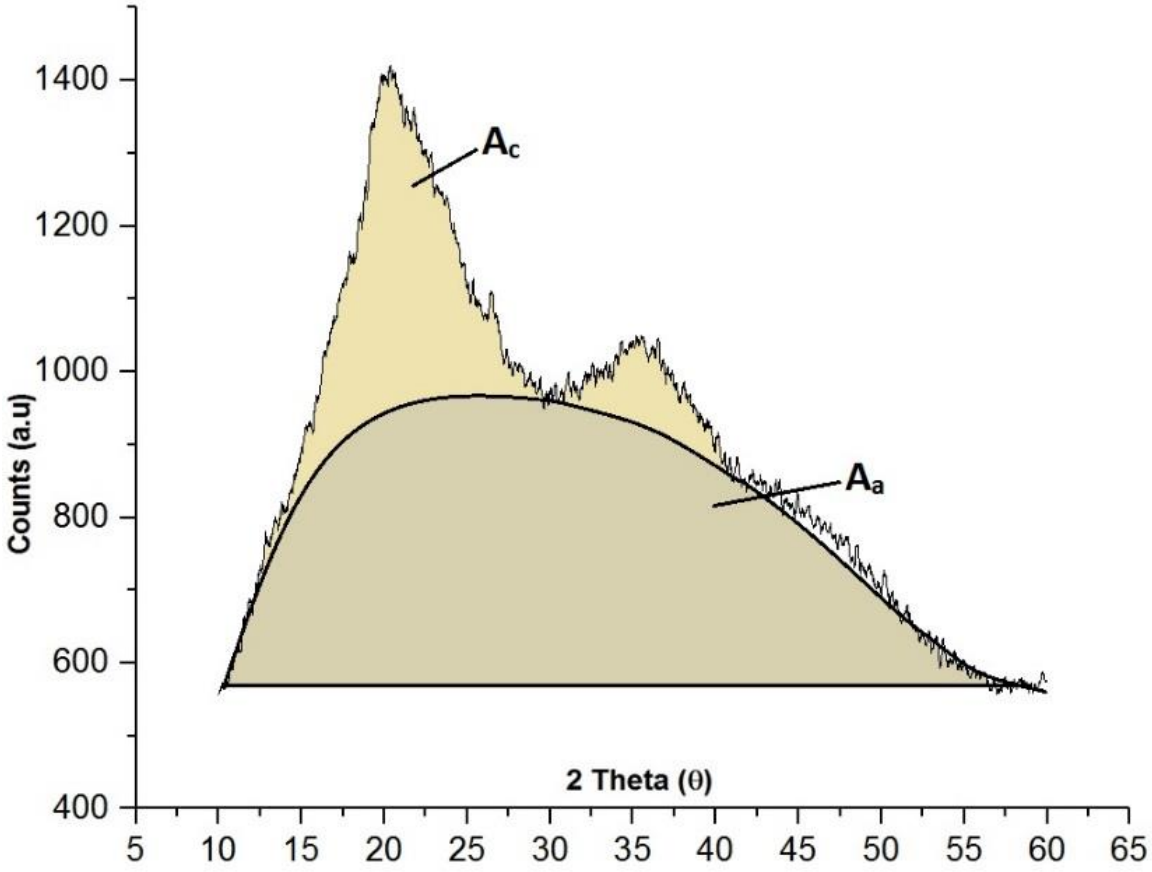


Figure 3-3 Representation of crystalline area (A_c) and amorphous area (A_a) in an XRD graph

3.2.16 Fourier transform infrared (FTIR) spectroscopy

Spectra pertaining to milk proteins and oat starch gels samples were obtained using the (ALPHA Bruker, Germany) FTIR spectrometer. The spectrum was recorded as the absorbance values derived from an average of 25 scans in the range of 600–4000 cm^{-1} with a resolution of 4 cm^{-1} taken at room temperature. The spectra were analysed by origin pro 8.5 software.

3.2.17 Gel Scanning electron microscopy (SEM)

Gel microstructure was studied on freeze-dried gel samples by using scanning electron microscope (JEOL, JSM-5410LV, Tokyo, Japan). Gel samples were prepared as described in pasting section 3.2.3, they were frozen in liquid nitrogen and finally freeze-dried. Freeze dried samples were fractured to expose the cross-section area and attached to the aluminium stub of the SEM with the help of a double-sided adhesive tape. After gold sputtering, the gel

samples were viewed under SEM at an accelerating potential of 15 kV.

3.2.18 Rheological properties

3.2.18.1 Steady state and dynamic rheology

All the rheological measurements were carried out in rheometer (MCR 302, Anton Paar, Austria). Starch pastes prepared as per the pasting section and were allowed to cool for 30 min to room temperature (25 °C) for further rheological measurements. A parallel plate geometry (PP50/SN) (d = 25 mm, gap 1 mm) was used, and temperature was kept constant at 25 °C for all measurements using Peltier element. The apparent viscosity (η) of oat starch WPI mixtures were recorded over a shear rate varied between 0.01 to 100 (1/s). To predict the flow curves (viscosity; versus shear rate) the Power Law equation was used:

$$\sigma = K\,(\dot{\gamma})^{n}$$

The Herschel-Bulkley model was used to describe the flow curves of oat starch /WPI gels.

$$\sigma = \sigma_0\, K\,(\dot{\gamma})^{n}$$

where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index (Pa s^n), n is the flow behaviour index (dimensionless) and σ_0 is the yield stress (Pa).

An amplitude strain- sweep test was performed at a constant frequency of 1 hz. During the test strain was varied from 10^{-2} % to 10^{3} %. Flow point (G'=G") was calculated by the Rheoplus (version 3.62) software. The storage modulus (G'), loss modulus (G'') and loss tangent (tan δ = G''/G') was calculated as a function of strain at 1hz. The overall resistance to deformation is calculated by complex modulus (G*) and is given by:

$$|G^*| = \sqrt{(G')^2 + (G'')^2}$$

Maximum strain (γ_{max}) and maximum stress (σ_{max}) at crossover (Flow point) were also calculated during amplitude sweep.

3.2.18.2 Thixotropy analysis

3.2.18.2.1 Hysteresis test

The oat starch/milk proteins gels were prepared, and the hysteresis loop was obtained by registering shear stress and the shear rates. The shear rate was increased from 1 to 50 s^{-1} in 3 min and then held constant at 50 s^{-1} for 1 min, followed by a decrease in shear rate back to 1 s^{-1} in 3 min. The experimental data was calculated for hysteresis area by Rheoplus software (version 3.62).

3.2.18.2.2 *Three interval thixotropy test (3ITT)*

A three-interval rotation (3ITT) step test was performed to break the structure of the gels formed in section 3.2.14 and to check its recovery after shear removal. In the first part, shear rate was kept constant for 30 sec at 1 s^{-1}, then gel structure was undergone breaking by ramping the shear rate to 100 s^{-1} for 60 s. In the final phase, the shear rate was kept constant at 1 s^{-1} for 210 s and structure recovery was calculated after 60 sec. Total time for the test was 300 s (5 min).

Recovery at 60 sec was calculated by following equation:

$$Rec_{60}\ (\%) = \left(\frac{\eta_{60}}{\eta^{0}}\right) \times 100$$

The difference ($\Delta\eta$) between initial viscosity (η_0) and final viscosity (η) was also calculated**.**

3.2.18.2.3 Time dependent

Time dependent behaviour of starch/WPI gels was analysed at constant shear rate of 50 s^{-1} for 5 minutes. Shear stress experimental data were fitted to the Weltman model and constant A and B are derived by using origin pro software (Ver. 8.5).

$$\sigma = A + B\ln t$$

Here, σ is the shear stress at time t (Pa), A is the initial shear stress (Pa), B is the time coefficient of thixotropic breakdown (Pa), and t is the time of shearing (s). The ratio ($\Delta\eta/\eta$) between initial viscosity (η_o) and final viscosity (η) was also calculated**.**

3.2.19 Statistical analysis

Statistical analysis was done using Minitab (Ver.17) statistical software for analysing the results obtained from three replications. The analysis of variance (ANOVA) and Tukey's test were used to analyse the results to compare differences among the mean values at $p < 0.05$ level of confidence. Rheological analysis was done by Rheoplus software (ver. 3.62). All experimental data fitted to different models by using Origin Pro software (ver. 8.5)

Chapter 4

4 The effects of dairy ingredients on the pasting, textural, rheological, freeze-thaw properties and swelling behaviour of oat starch

- This part has been published in Food Chemistry (Kumar, L., Brennan, M., Zheng, H., & Brennan, C.; 2018). The effects of dairy ingredients on the pasting, textural, rheological, freeze-thaw properties and swelling behaviour of oat starch. *Food Chemistry*, *245*, 518–524.

4.1 Introduction

Oats belong to the genus *Avena* and are considered a minor cereal crop based on annual production. In spite of a low level of manufacture, oat starch attracts most attention among cereal starches. Oat starch exhibits typical properties including small granule size, a well-developed granule surface, and high lipid content (Hoover et al., 2003; Mirmoghtadaie et al., 2009). Proteins used in fortification of foods include plant proteins (soy, wheat, rice, corn and other plant sources), milk proteins, egg proteins, meat proteins and fish proteins. Each type of protein exhibits different functional properties and will have different applications in different types of food products. Various studies have been conducted with quality protein fortification to high carbohydrate foods to produce wholesome foods for the endorsement and maintenance of good health (Ainsworth, Ibanoğlu, Plunkett, Ibanoğlu, & Stojceska, 2007; Amaya-Llano, Hernández, Tostado, & Martínez-Bustos, 2007; Cho & Rizvi, 2010). Starch is a polysaccharide with extensive applications in food technology, due to its texture enhancing properties, its ready availability and low cost. Despite the industrial significance of starch-milk protein mixtures, little fundamental research is available on oat starch-milk protein interactions. It has been shown that concentration, botanical origin, composition and processing history are critical in understanding the changes occurring in the starch-milk

protein systems (Corredig, Sharafbafi, & Kristo, 2011). However, there are no studies on oat starch interaction with milk proteins and in systems in which milk proteins and oat starch co-exist, it is important to understand the mechanisms, interactions and synergistic effects that can provide maximum benefit to oat-milk based food products.

Hence, the aim of this study was to investigate the effect of milk components on oat starch properties. This study also aimed to show that how protein-starch interactions affect functional properties of foods, these functional properties also depend on the source of starch and the amount and type of milk components. This knowledge will be useful to manufacturers who may seek to enhance food texture by blending different proteins and will be helpful for various dairy and cereal products manufacturing industry as bakery products, breakfast foods, dairy drinks and RTEs etc. during processing.

4.2 Material and Methods

4.2.1 Materials

This section is described in 3.1.

4.2.2 Sample preparation

The mixtures with oat starch and milk ingredients were prepared as 100:0, 95:5 and 90:10 with WPC, WLAC and SMP respectively (w/w). In the pasting and textural studies, starch suspension concentration was taken at 10.71% (w/w) while during freeze-thaw andswelling powerit was 5% (w/w). In the solubility index and WBC determinations starch suspension concentration was 1% and 6.66 % (w/w) respectively. All experiments were conducted in triplicate.

4.2.3 Starch extraction

This section is described in 3.2.1

4.2.4 Physicochemical analysis of oat starch

This section is described in 3.2.2

4.2.5 Pasting properties

This section is described in 3.2.3

4.2.6 Water binding capacity (WBC)

This section is described in 3.2.4.

4.2.7 Swelling power and solubility index

This section is described in 3.2.5

4.2.8 Freeze-thaw stability

This section is described in 3.2.7

4.2.9 Textural properties

This section is described in 3.2.12.1

4.2.10 Colour

This section is described in 3.2.8

4.2.11 Flow Characteristics

This section is described in 3.2.9

4.2.12 Microscopy

This section is described in 3.2.10.

4.2.13 Statistical analysis

This section is described in 3.2.19

4.3 Result and Discussion

4.3.1 Starch isolation and physiochemical properties of oat starch

The results of yield and composition of oat starch and milk components are presented in Table 4-1. The yield of prime starch recovered from oat flour was 47±4%, which is in agreement with previously published literature range of 48.5-61% (Paton 1977). The protein content of the purified starches was 0.5% (dry basis), indicating that protein was removed. The lipid content was found to be 0.8%, which lies in the previous observations of oat starch lipid range of 0.7-2.5% (Hoover *et al.* 2003) . Starch content of extracted starch was found to be 91%, which indicates high starch purity (Shah, Masoodi, Gani, & Ashwar, 2016a). The protein content of Milk powders was found to be 77.5%, 79.2% and 32.6% for WPC, WLAC and SMP respectively.

Table 4.1 Characteristics of oat starch

Characteristics of oat starch	**Composition**
Moisture (%)	9±0.5
Fat (%)	0.8±0.1
Protein (%)	0.5±0.05
Extraction rate/ Yield (%)	47±4
Starch content (%)	91±2
Swelling power at 95 °C **(g/g)**	10.17± 0.09
Solubility Index (%)	21.11± 0.00
Paste clarity	Opaque

4.3.2 Determination of pasting properties

The RVA paste properties of starch-milk component system is presented in Table 4.2. The peak viscosity of control oat starch was found to be medium-low similar to wheat amongst cereal starches (Hoover et al., 2003). However, the high setback viscosity is a characteristic of oat starch, leading to the onset of a thick gel. The RVA curves generally exhibited the same typical RVA starch pasting curve trend, namely that the viscosity (peak, trough, breakdown, final and

setback) was decreased by substitution of oat starch with milk components. The results illustrate that WPC and SMP generally showed a significant influence on RVA parameters than WLAC. The results displayed in Table 4.2 appear to be following the same trend to those observed by Carvalho et al., (2007); Sopade, Hardin, Fitzpatrick, Desmee, & Halley, (2006), who measured a decrease in viscosity when protein content (casein or WPI) in starch-based products was substituted. The same typical RVA curve was also observed in the case of rice flour substituted with rice protein isolate (Kim et al., 2014). The higher the milk component content, the lower were the peak, final and trough viscosities as well as the setback values. This possibly confirms that starch dominated network is more prevailing at low level of milk constituents and no phase transition was observed in the study. Generally, starch forms a viscous gel, and the whey proteins aggregates could have acted as a filler when the starch concentration was high (Noisuwan et al. 2009). The peak viscosity may also be attributed to the water absorption, swelling power of starch molecules and water holding capacity of the gel, while the measured reduction in the peak viscosity with an increase in milk component might suggest poor water holding capacity in starch-milk protein system when compared to starch gels alone (Sopade et al., 2006). WPC and SMP showed higher peak viscosity in comparison to WLAC at both 5% and 10% concentration. This trend might be due to the WLAC composition which has higher α- lactalbumin to β-lactoglobulin ratio in comparison with WPC, α- lactalbumin is more resistant to denaturation than β-lactoglobulin resulting in insoluble proteins, hence can phase separate easily (Wijayanti, Bansal, & Deeth, 2014a), while WPC is more soluble and can contribute to viscosity (Onwulata, Konstance, Cooke, & Farrell, 2003). SMP contains more lactose, salts and casein protein than WPC and WLAC, showed a higher peak viscosity in comparison with other milk components at low concentration and almost equal to WPC at 10%. This effect is not fully understood yet, it is probably due to particle

rigidity of starch in presence of skim milk powder (Abu-Jdayil, Mohameed, & Eassa, 2004; Matser & Steeneken, 1997). The paste stability of the starch mixtures could be expressed by either breakdown viscosity or stability ratio. The paste stability explained the hydration, starch swelling power and shear resistance of starch paste during heating. Low breakdown viscosity and high stability ratio often associated with low hydration and swelling power, and high shear resistance (Shafie, Cheng, Lee, & Yiu, 2016). The Stability ratio was increased when starch was substituted with different milk components, which indicated less starch swelling and less breakdown of starch granules. SMP significantly increased the stability ratio more than WPC and WLAC, which was due to presence of lactose, caseins and minerals in skim milk powder. Caseins can also reinforce the starch granule structure and might adsorb on the granule surface by the capability of self-association to form micelles, hence restricts starch swelling and breakdown resulting in increased stability (Kumar et al., 2017). Setback viscosity and setback ratio indicated starch retrogradation tendency after gelatinization and cooling at 50°C. At 5% substitution of starch with WPC and WLAC; there was no significant difference in setback ratio to control sample except SMP. SMP showed lower setback ratio at 5% and 10% substitution than WPC, WLAC respectively. Moreover, WPC and WLAC showed significant decreases in setback ratio and setback viscosity at 10% substitution of starch. SMP affected the setback ratio most and indicated less restructuring of amylose molecules. Relative breakdown indicated the extent of disruption of starch components in presence of milk ingredients. Initial rise in viscosity occurred when starch granules and proteins began to absorb water and swelled as the temperature increased gradually, this temperature was known as pasting temperature and related with gelatinisation properties of starch. As it can be depicted from Table 4.2, SMP showed a significant increase in pasting temperature at 10% substitution concentration. The higher pasting temperature indicated the resistance potential

against swelling in the ingredient. Noisuwan et al., (2007, 2008) reported the same behaviour of SMP on gelatinization temperature.

Table 4.2 Pasting characteristics of oat starch with milk components

Samples	Peak Viscosity* (cP)	Trough Viscosity* (cP)	Breakdown Viscosity* (cP)	Final Viscosity* (cP)	Setback Viscosity* (cP)	Stability Ratio	Setback ratio	Relative breakdown	Pasting Temperature (°C)
Oat Starch	2784.50±24.80^{a}	2092.50±3.48^{a}	692.00±21.30^{a}	4613.00±19.80^{a}	2520.50±23.30^{a}	0.75±0.00^{d}	2.20±0.01^{b}	0.274±0.01^{a}	93.02±0.10^{b}
OS+WPC[2] 5%	2187.50±23.30^{c}	1813.50±20.40^{b}	373.98±2.80^{b}	3921.00±17.00^{b}	2107.50±3.48^{b}	0.82±0.00^{c}	2.16±0.01^{bc}	0.177±0.00^{b}	93.75±0.00^{ab}
OS+WLAC[3] 5%	2103.00±18.30^{d}	1732.98±1.44^{c}	370.00±17.00^{bc}	3765.50±19.10^{c}	2032.50±20.40^{b}	0.82±0.00^{c}	2.17±0.01^{bc}	0.182±0.01^{b}	92.12±1.09^{b}
OS+SMP[1] 5%	2367.48±13.41^{b}	2045.00±26.90^{a}	322.50±13.49^{c}	3907.02±1.44^{b}	1862.00±28.30^{cd}	0.86±0.00^{b}	1.91±0.02^{d}	0.173±0.00^{b}	94.12±0.60^{ab}
OS+WPC 10%	1941.50±21.90^{e}	1612.98±11.29^{d}	328.50±10.61^{bc}	3452.00±14.20^{d}	1839.00±25.50^{d}	0.83±0.00^{c}	2.14±0.02^{c}	0.178±0.00^{b}	93.77±0.03^{ab}
OS+WLAC 10%	1668.48±0.68^{f}	1419.54±6.36^{e}	249.00±7.13^{d}	3352.02±11.29^{e}	1932.48±4.92^{c}	0.85±0.00^{b}	2.36±0.00^{a}	0.128±0.00^{c}	92.60±0.56^{b}
OS+SMP 10%	1951.02±2.80^{e}	1734.00±5.60^{c}	217.02±2.80^{d}	3248.00±21.20^{f}	1514.00±15.50^{e}	0.88±0.00^{a}	1.87±0.00^{d}	0.143±0.00^{c}	95.30±0.07^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

4.3.3 Water binding capacity (WBC) analysis of oat starch/milk protein ingredients mixture

Water binding capacity of oat starch-milk components ranged from 100% - 121% (Table 4.3). WBC of oat starch increased significantly when starch was replaced with milk components. All the milk ingredients showed an increase in WBC in comparison to control, however there was no significant difference observed in between each of them at 5% and 10%. An increase in WBC is a result of degree of availability of the water binding sites of oat starch and native water absorption tendency of different milk components. The substitution of starch with milk components increased WBC significantly, which can be attributed to physical adsorption of proteins on starch granules via hydroxyl groups and inter-glucose atoms binding sites (Considine et al., 2011; Sandhu & Singh, 2007).

4.3.4 Determination of swelling Power and Solubility Index

Swelling tests are simple analyses that measure the uptake of water during the gelatinization of starch. Swelling power and solubility index were presented in Table 4.3. Swelling of starch granules were not significantly affected by milk proteins including WPC and WLAC. However, SMP has significant effect on starch swelling power. As discussed in section 4.3.2, presence of salts, minerals, lactose and caseins in SMP hindered the starch swelling which resulted in less starch swelling (Noisuwan et al. 2007).

The Solubility index indicates the leaching behaviour of starch during gelatinization. Solubility index increased as starch was substituted with milk components. However, here it should be taken in to account that milk proteins and SMP are soluble in water. So, this solubility index does not indicate true starch molecules leaching. SMP had the lower solubility index among all the milk components, as SMP restricted starch swelling and breaking, which finally resulted in less solubility due to less leached amylose molecules.

Table 4.3 Physicochemical and textural properties of oat starch system containing milk components

Samples	Swelling power (g/g)	Solubility Index (%)	Hardness (N)	WBC (%)
Oat Starch	10.17± 0.20^{ab}	21.11± 1.92^{c}	0.42±0.01^{a}	100.0±1^{c}
OS+WPC[2] 5%	10.51± 0.21^{ab}	30.41± 2.03^{ab}	0.37±0.01^{bc}	111.23±3.38^{b}
OS+WLAC[3] 5%	10.52± 0.27^{ab}	30.41± 2.03^{ab}	0.33±0.01^{de}	109.12±3.38^{b}
OS+SMP[1] 5%	9.05± 0.30^{c}	26.90± 2.03^{bc}	0.41±0.02^{ab}	115.08±1.60^{ab}
OS+WPC 10%	10.80± 0.14^{a}	30.86± 2.14^{ab}	0.31±0.02^{ef}	121.85±2.80^{a}
OS+WLAC 10%	10.11± 0.11^{b}	34.57± 2.14^{a}	0.28±0.01^{f}	119.26±2.57^{a}
OS+SMP 10%	8.31± 0.34^{d}	27.78± 2.62^{b}	0.35±0.01^{cd}	115.56±2.22^{ab}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**
[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

4.3.5 Freeze-thaw stability analysis

Freeze-thaw stability is a key characteristic of starch that is used to determine the structural strength or textural resistance to undesirable temperature shocks occurring during freezing and thawing. Syneresis is a phenomenon of water separation from a gel structure due to shrinking of gel network by the restructuring of leached starch molecules. However, syneresis is an undesirable phenomenon for starch gels or starch containing products as it is related to product deterioration. The control oat starch sample showed a syneresis of 15.66% after first cycle and it indicated the maximum stability compared to substitution with milk components (Table 4.4). All the substituted samples increased syneresis after consecutive freeze thaw cycles. During freezing of starch gels, phase formation occurs between starch molecules and ice crystals. Therefore, upon thawing, melted water can be easily expressed from the amylose/amylopectin network in the form of syneresis (Muadklay & Charoenrein, 2008).

Substitution of starch with milk components increased syneresis of gels as starch network became weak due to low level of starch fraction, and reduced its water holding capacity. SMP significantly increased the syneresis rate in comparison with milk proteins, it might be due to less water holding capacity of SMP components in comparison with proteins. Another reason could be due to less relative breakdown of starch granules which resulted in less amylose leaching in the matrix, leading to a weak gel network.

4.3.6 Gel textural analysis

The gel hardness of different Oat starch-milk components gels was determined using texture analyser are shown in Table 4.3. Gel formation is predominantly affected by amylose gelation rather than long term amylopectin retrogradation. The gel hardness of oat starch was significantly higher than other samples. As the starch content decreased, gel strength weakened significantly ($p<0.05$). A starch gel is consisted of deformed swollen starch molecules and leached amylose in the continuous matrix, these structural parameters significantly affects firmness of starch gels. SMP showed a more gel hardness of Oat starch with 5% and 10% SMP fraction in comparison to WPC and WLAC. This could be attributed to presence of casein micelles, soluble milk minerals and lactose, which can provide more junction jones for intermolecular association of amylose continuous network (Gunaratne, Ranaweera, & Corke, 2007). Berski et al., (2016) also reported an increase in gel hardness of oat flour gels in the presence of sugars. WPC at 10% and 5% concentration showed a high gel hardness trend in comparison with WLAC but a significant difference was observed at only 5% concentration. This may be due to more β-lactoglobulin content and capacity to form whey aggregates, while WLAC had inability to form aggregates due to high α- lactalbumin to β-lactoglobulin ratio than WPC.

Table 4.4 Syneresis and colour values of oat starch gel containing milk components

Samples	1st Day (%)	3rd Day (%)	5th Day (%)	L*	a*	b*	ΔE
Oat Starch	15.66±0.87^{b}	16.50±0.95^{c}	18.70±0.08^{bc}	92.66±0.37^{abc}	-9.96±0.02^{bcd}	17.70±0.05^{d}	0±0^{c}
OS+WPC[2] 5%	17.95±0.85^{ab}	18.30±0.58^{abc}	16.45±0.12^{c}	93.53±0.43^{a}	-9.74±0.09^{b}	18.25±0.12^{bc}	1.06±0.07^{b}
OS+WLAC[3] 5%	17.30±0.75^{ab}	17.50±0.24^{bc}	19.22±0.01^{b}	93.20±0.19^{ab}	-8.23±0.06^{a}	17.51±0.13^{d}	1.86±0.12^{a}
OS+SMP[1] 5%	18.49±0.33^{a}	18.43±0.56^{abc}	21.91±0.07^{a}	92.38±0.28^{bc}	-10.15±0.02^{cd}	17.93±0.02^{cd}	0.63±0.28^{b}
OS+WPC 10%	17.93±0.61^{ab}	18.85±0.68^{ab}	17.42±0.82^{bc}	92.92±0.10^{abc}	-9.88±0.03^{bc}	18.60±0.06^{b}	0.99±0.16^{b}
OS+WLAC 10%	18.24±0.90^{ab}	17.67±0.06^{abc}	19.23±0.66^{b}	92.81±0.47^{abc}	-9.79±0.09^{bc}	19.55±0.14^{a}	1.87±0.14^{a}
OS+SMP 10%	19.42±0.38^{a}	19.79±0.13^{a}	22.70±0.76^{a}	92.15±0.15^{c}	-10.29±0.35^{d}	19.45±0.32^{a}	1.90±0.18^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

4.3.7 Colour

Colour of starch gels were measured for visual appearance, there was no significant difference observed between control and substituted samples in terms of L* (Lightness) and -a* (Greenness) values (Table 4.4). SMP normally reflects a greenish-yellowish colour as a dry ingredient, therefore, it increased the negative values of a* and b* at 5% and 10% substitution. Similarly, milk proteins reflect a yellowish colour, which was observed as an increase in b* (yellowness) in comparison with oat starch alone.

The total colour difference was found to be more prominent at 5%, 10% WLAC and 10% SMP. However, there was no difference in WPC containing gels at 5% and 10% concentration. Overall, visual appearance of oat starch gels containing milk components appeared to be yellowish in colour in comparison with oat starch gel alone.

4.3.8 Flow characteristics

Viscosity decreased as the shear rate varied between 1.29 s^{-1} to 516 s^{-1}. Substitution of oat starch with milk proteins (WPC and WLAC) and SMP decreased the viscosity of the system in comparison with oat starch alone but supported the same shear thinning behaviour, where the apparent viscosity decreases with shear rate (Olsson et al. 2000; Quiroga and Bergenståhl 2008). SMP substitution reduced the apparent viscosity significantly and showed reduced shear thinning behaviour in comparison with dairy whey containing proteins. This could be due to reduced swelling of starch granules in presence of skim milk powder and reduced leaching of amylose, resulting in less viscosity. The effects of milk components on the flow curves of oat flour were quantified using the power law model, which fits well the η versus γ data for Oat starch paste (Table 4.5). R^2 values (0.94-0.97) showed good fit of power law model to experimental data. The obtained power law model parameters reported in Table 4.5. A Newtonian fluid has the flow behaviour index (n)= 1, and lower 'n' value indicates a high

pseudoplastic behaviour of starch paste (Lu, Luo, & Xiao, 2012; Pongsawatmanit, Temsiripong, Ikeda, & Nishinari, 2006). Consistency coefficient, K, from the power model reflects magnitude of viscosity in terms of consistency (Sikora, Kowalski, Tomasik, & Sady, 2007). The Flow behaviour index was significantly increased with the addition of SMP, however milk proteins did not show any significant effect. As it can be shown from the results, oat starch substitution with 10% WPC and WLAC decreased the consistency coefficient (K) significantly to control sample, which is a measure of the shear viscosity. SMP had shown a more prominent effect on the consistency coefficient (K) in comparison with milk proteins at both 5% and 10% substitution levels.

Table 4.5 Flow behaviour parameters of oat starch paste and effect of thermal processing on starch granule size containing milk components

Samples	Flow index (n)	Consistency Index (K)	R^2	Starch granule size (µm)	Range (min.-max)
Oat Starch	0.311±0.011^{b}	6.84±0.51^{a}	0.968±0.003	10.36±2.66^{a}	6.62-16.78
OS+WPC[2] 5%	0.279±0.004^{b}	6.21±0.20^{ab}	0.970±0.001	9.58±1.78^{ab}	4.43-12.92
OS+WLAC[3] 5%	0.296±0.008^{b}	6.71±0.40^{ab}	0.978±0.004	9.432±2.37^{ab}	5.30-14.54
OS+SMP[1] 5%	0.398±0.012^{a}	2.96±0.26^{d}	0.959±0.009	8.08±1.65^{bc}	5.08-10.97
OS+WPC 10%	0.293±0.014^{b}	5.16±0.44^{c}	0.977±0.003	8.49±1.87^{bc}	5.19-11.58
OS+WLAC 10%	0.285±0.004^{b}	5.78±0.30^{bc}	0.977±0.003	9.15±2.83^{ab}	4.98-16.76
OS+SMP 10%	0.386±0.020^{a}	2.15±0.27^{d}	0.940±0.014	7.1±1.45^{c}	4.0-9.78
Starch granule morphology					
Granule shape	Polygonal/polyhedral to irregular				
Granule size (µm)	4.80±1.35			2.33-7.67 (Range)	

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

4.3.9 Microscopy

4.3.9.1 Thermal processing effect on granule size

Oat starch granules tend to exist in clusters of individual granules. The granules of all starch particles ranged from irregular to polygonal in shape with average granule diameter of 4.9±1.43 µm within a range of 2.37- 9.27 µm (Figure 4.1 a,b). The effect of thermal processing on starch granule in presence of milk components was measured by light microscopy. Size of oat starch granules were decreased significantly at 10% SMP, WPC and 5% SMP substitution of oat starch (Table 4.5). However, SMP showed a more size reduction trend over WPC. Granule size reduction by SMP can be influenced by various components present in it. Milk minerals contain a number of highly charged ions, such as Ca^{2+}, Mg^{2+}, PO_4^{3-} and citrate (Oh, Anema, Pinder, & Wong, 2009). It is possible that the highly charged ions in skim milk minerals reduced the amount of free water available for starch gelatinisation, thereby contributing to the observed retardation of granule size (Chiotelli, Pilosio, & Le Meste, 2002). Oh *et al.*, (2009) also reported that skim milk components can affect starch gelatinization by interaction of lactose with starch molecules in the amorphous or crystalline regions, to stabilize the structure, would slow down the progress of gelatinisation. In summary, the observed reduction of starch granule size in 5% and 10% SMP (Table 4.5) was attributed to the combined effect of caseins, soluble milk minerals and lactose.

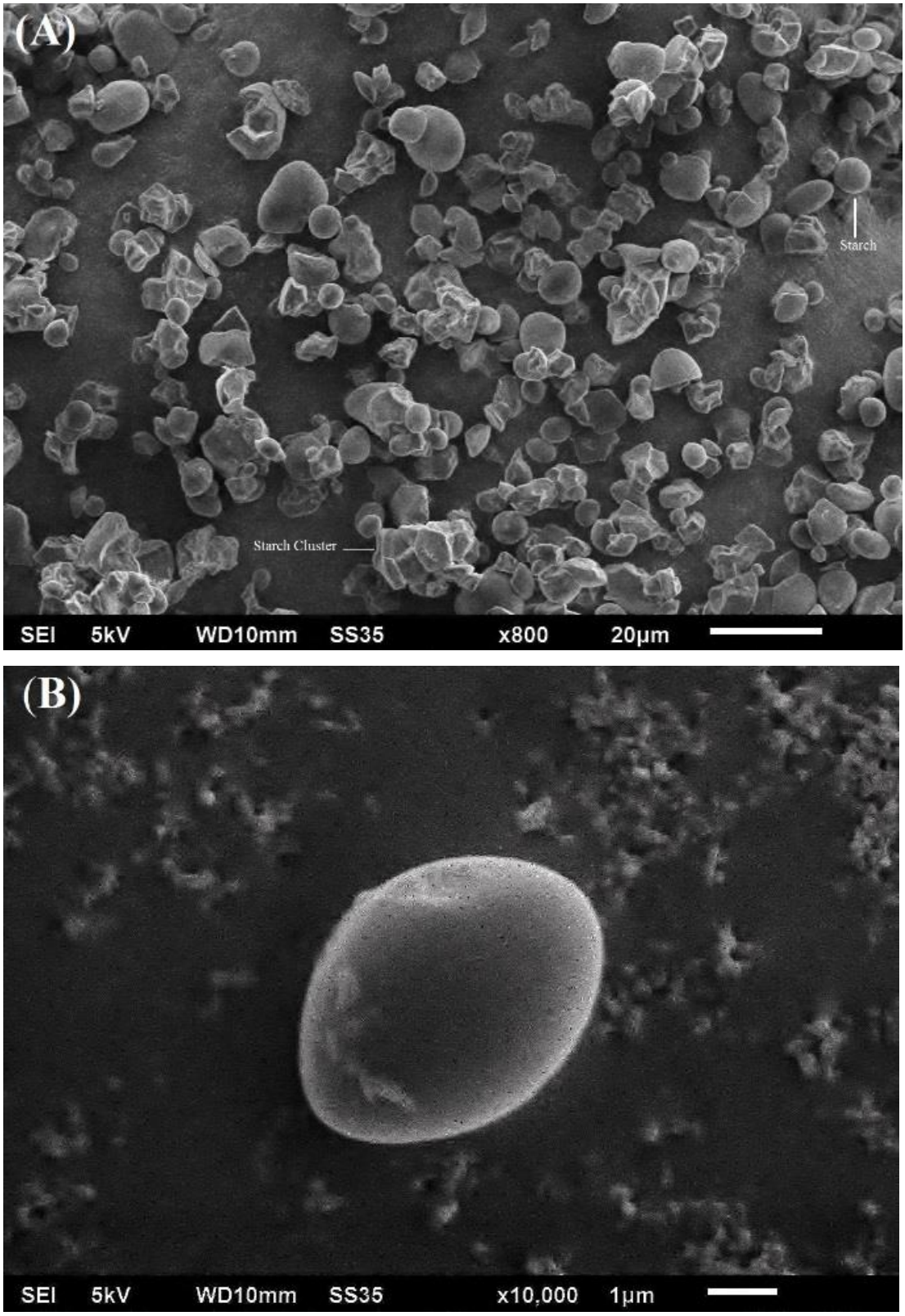


Figure 4-1 Oat starch granule morphology

4.4 Conclusion

The substitution of the oat starch with three different milk components, WPC, WLAC and SMP, decreased the viscosity of oat starch when heated and cooled in water. Pasting properties revealed that hot paste stability was increased when milk components were added. The addition of SMP had a greater effect on paste stability, starch swelling and granule size than WPC and WLAC. Furthermore, SMP increased the freeze-thaw stability, while viscosity was decreased. Finally, although further investigations are required to fully understand the mechanisms by which milk components interact with oat starch, the trends obtained are valuable in developing novel food products and process optimisation. This study suggested that at high starch concentrations, starch dominates the viscous network and low-level milk protein substitution might help to improve or stable food product quality except SMP, depending upon desired product type such as porridge, yoghurt and sports gel. The findings of this study explained indirectly why the small amount of starch substitution with milk components can affect the pasting behaviour which ultimately can affect food product texture and overall acceptability.

Chapter 5

5 Effect of milk components on pasting, hydration, syneresis and large deformation viscoelastic properties of oat flour

5.1 Introduction

In the previous chapter, the effect of different milk protein ingredients on oat starch is investigated and it was observed that milk protein ingredients have a prominent effect on oat starch. Furthermore, to elucidate the effects of these ingredients on oat flour this study was examined, as oat flour consist of oat starch, fat, proteins and fibre, which can result in different trend than pure starch.

Currently there is an increasing demand for nutritionally balanced foods because of the increasing risk of diabetes and obesity. Oats (*Avena sativa*) belongs to the grass family Poaceae and are one of the most valuable cereal grains as they are rich source of dietary fiber in particular β-glucan. Because of this, oats have attracted positive consumer attention (Shah, Masoodi, Gani, & Ashwar, 2016b; Weightman et al., 2004). The protein content of oats ranges from 9-15% with a higher concentration of lysine than other cereals; oats has almost 2-8% of β-glucan as dietary fibre and 3-11% of lipid content, which is higher than other cereals (Rasane, Jha, Sabikhi, Kumar, & Unnikrishnan, 2015). However, oats lack high-quality protein sources, which is quite common among other cereal sources. Therefore, high-quality source proteins from milk, meat, egg and fish can be added to foods for developing more nutritionally balanced food. Milk proteins are classified into casein and whey, in which, whey proteins consist of almost 20% of total protein. Whey proteins have antioxidant, immunostimulatory, anticarcinogenic properties and provide high-quality protein for nutrition, which makes them an excellent source for fortifying food products (Hoppe et al., 2008). Most commonly, oats are consumed as oatmeal/porridge, but have been used in a variety of foods such as baked goods,

granola bars, infant foods and dairy and non-dairy food products (Zhu, 2017) and are becoming more popular as a food ingredient in the food industry (Butt et al., 2008).

Hence, oat flour components such as starch, β-glucan, protein and lipids can interact with milk components in milk protein fortified systems, altering the inherent properties of oat flour. Oat starch, which is a major part of flour contributes to the overall behaviour of oat flour. Therefore, most of the research is done on isolated starches with milk proteins and much less on integral flours (Kumar et al., 2018; Angkana Noisuwan et al., 2008). There are no studies on oat flour interaction with milk proteins in a system, where milk proteins can affect native flour properties. However, β-glucan showed thermodynamic incompatibility with milk proteins, which can influence stability, texture and viscoelastic characteristics of food products (Sharafbafi, Tosh, Alexander, & Corredig, 2014). The formation of amylose-lipid complexes can also be formed during thermal processing of flour or starch containing lipids, leading to affect native physical properties of oat flour (Mayachiew, Charunuch, & Devahastin, 2015).

In the experiments reported in this chapter, large strain mechanical tests were applied to evaluate the viscoelastic and textural behaviour of oat flour/milk proteins gels and paste matrices. Large deformation mechanical tests mimic industrial processing of semi-solid foods, which can also be used to determine rheological characterization and correlation with sensory attributes (Bollaín, Angioloni, & Collar, 2005; B. S. Roopa & Bhattacharya, 2008).

Hence, the present work was undertaken to study pasting, hydration, viscoelastic and syneresis characteristics of oat flour as affected by milk components; this information could be essential for the development of new oat-dairy based new products and adjustment of processing parameters. This study can help to utilize oat flour and to understand the usage of milk components in more complex food systems. Also, the research output will be beneficial for manufacturing of bakery foods, dairy and non-dairy food products, sauces and ready to

eat snacks.

5.2 Material and Methods

5.2.1 Materials and sample preparation

This material section is described in 3.1. The mixtures with oat flour and milk ingredients were prepared as 100:0, 95:5 and 90:10 with WPC, WLAC and SMP respectively (w/w). In the pasting and penetration textural studies, oat flour suspension concentration was taken at 10.71% (w/w) while during hydration, back extrusion properties and syneresis, it was 10% (w/v) and 5% (w/w) respectively. All experiments were conducted in triplicates.

5.2.2 Pasting properties

This section is described in 3.2.3

5.2.3 Hydration properties (Water absorption index and Water solubility index)

This section is described in 3.2.6

5.2.4 Syneresis

This section is described in 3.2.11

5.2.5 Large deformation viscoelastic properties

This section is described in 3.2.12

5.2.5.1 Penetration mechanical test

This section is described in 3.2.12.1

5.2.5.2 Back extrusion mechanical test

This section is described in 3.2.12.2

5.2.6 Statistical analysis

This section is described in 3.2.19

5.3 Results and Discussion

5.3.1 Pasting properties

The pasting properties of oat flour were analysed by RVA as shown in Table 5.1. The higher the oat flour content substitution with milk components, the lower peak, final, trough, setback and total viscosities were observed. The peak viscosity decreased as oat flour was substituted with milk components, which was more prominent at 10% substitution. The peak viscosity is related to the degree of swelling of starch granules during heating and water absorption by flour (Sopade et al., 2006). SMP fortified samples showed a higher peak viscosity in comparison to WLAC enriched sample at 5%; the same trend showed for both WPC and SMP enriched samples where the enrichment increased to 10 % substitution (P<0.05). It is generally understood that the protein concentration determines the peak viscosity of starch paste (Considine et al., 2011). However, such understanding does not explain the current results in which WLAC enriched samples had lower peak viscosities comparing with the paired samples containing SMP. It is important to point out that the detailed mechanism about how different dairy ingredients affect peak viscosity of oat flour paste is unknown. The current results emphasized the complexity of the interaction mechanism between milk solids and oat flour components. Noisuwan et al. (2008) pointed out the added protein type, and concentration determine the peak viscosity of starch paste system. Also, whey protein denaturation increased the peak viscosity of rice starch system. Therefore, it is reasonable to speculate that the lower peak viscosity of α- lactalbumin (WLAC ingredient) enriched oat flour system is due to its less formation of denaturation induced protein aggregates comparing with other dairy ingredients fortified samples. As casein facilitates the interaction of α-lactalbumin and β-lactoglobulin (SMP containing sample) and β-lactoglobulin determines the degree of whey protein complex formation (Elfagm & Wheelock, 1978; Schokker, Singh, & Creamer, 2000). The current results suggest that the protein type/composition in dairy ingredient rather than protein concentration or non-protein solids governs the peak viscosity of oat starch/flour

paste system containing dairy components. For the same quantity of substituted milk powder solids, SMP contains amounts of lactose, minerals and casein proteins compared with other two dairy ingredients. Previous research has shown that addition of low molecular weight sugars affects the gelatinisation of starch granules with elevating gelatinisation temperature (Perry & Donald, 2002). Moreover, minerals were found to increase the gelatinization temperature at a relatively lower concentration (< 1M) but to decrease the gelatinization temperature when the concentration further increased (>1M) (Evans & Haisman, 1982). Such research findings explain the higher pasting temperature of SMP enriched samples at both 5% and 10% substitution levels as results in the current research (P<0.05).

The paste stability of the flour mixtures could be expressed by either breakdown viscosity or stability ratio. The paste stability explained the shear resistance of oat flour paste during heating, the highest value of breakdown viscosity was found in SMP and lowest in WPC and WLAC at 5% and 10% oat flour substitution, suggesting that WPC and WLAC substitution could make most stable hot paste than SMP. High breakdown viscosity or stability ratio indicates lower power to withstand high shear resistance and often associated with hydration and swelling power (Adebowale, Olu-Owolabi, Olawumi, and Lawal, 2005; Shafie et al., 2016).

Table 5.1 Pasting characteristics of oat flour with milk components

Samples	Peak Viscosity* (cP)	Trough Viscosity* (cP)	Breakdown Viscosity* (cP)	Final Viscosity* (cP)	Setback Viscosity* (cP)	Stability Ratio*	Setback ratio*	Relative breakdown*	Pasting Temperature (°C)*
Oat flour	1466.5±14.50^{a}	1262±12.00^{a}	204.5±2.50^{a}	2547±24.00^{a}	1285±12.00^{a}	0.86±0.00^{d}	2.01±0.00^{de}	0.15±0.00^{a}	90.55±0.00^{b}
OF+WPC[2] 5%	1116±58.00^{bc}	983±88.00^{b}	133±30.00^{bc}	2038.5±132.50^{b}	1055.5±44.50^{bc}	0.87±0.00^{cd}	2.07±0.05^{cd}	0.12±0.00^{ab}	90.47±0.02^{b}
OF+WLAC[3] 5%	1088.5±8.50^{c}	986.5±4.50^{b}	102±13.00^{c}	2133.5±10.50^{b}	1147±6.00^{b}	0.90±0.01^{bc}	2.16±0.00^{b}	0.08±0.01^{bc}	90.10±0.40^{b}
OF+SMP[1] 5%	1158.5±2.50^{b}	1009.5±18.50^{b}	149±21.00^{b}	2125.5±26.50^{b}	1116±8.00^{b}	0.87±0.01^{cd}	2.10±0.01^{bc}	0.13±0.01^{ab}	91.70±0.35^{a}
OF+WPC 10%	979.5±7.50^{d}	934.5±4.50^{b}	45±12.00^{d}	1878±19.00^{c}	943.5±14.50^{d}	0.95±0.01^{a}	2.00±0.01^{e}	0.04±0.01^{c}	90.50±0.00^{b}
OF+WLAC 10%	784.5±22.50^{e}	735.5±21.50^{c}	49±1.00^{d}	1719.5±38.50^{d}	984±17.00^{d}	0.93±0.00^{ab}	2.33±0.01^{a}	0.04±0.00^{c}	90.50±0.40^{b}
OF+SMP 10%	923.5±4.50^{d}	828.5±11.50^{c}	95±7.00^{c}	1785±22.00^{cd}	956.5±10.50^{d}	0.89±0.00^{bcd}	2.15±0.00^{b}	0.09±0.00^{b}	92.05±0.00^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

During the setback viscosity phase, cooling causes the re-association of starch molecules (especially linear chains such as amylose) which results in gel structure formation and an increase in final viscosity due to retrogradation; thus, a lower setback value is indicative of low retrogradation. Substitution of oat flour with milk components reduced the setback significantly. However, there is no significant difference between each milk component at the same respective substitution of oat flour. An initial rise in viscosity occurred when starch granules and proteins began to absorb water and swelled as the temperature increased gradually (Batey, Crosbie, & Ross, 2007). The temperature at this point, known as the pasting temperature, was associated with gelatinization properties of the starch present in flour. As depicted in table 5.1, SMP increased the pasting temperature significantly at 5% and 10% substitution (P<0.05). Noisuwan et al. (2008, 2007) showed the same behaviour of SMP on gelatinization temperature. In the previous chapter, the same behaviour was observed of SMP on oat starch, hence it can be suggested by this study that SMP has a strong effect on thermal properties of oat starch irrespective of presence of other oat flour components such as fibre, fat and protein. This pasting study indicates that the flour dominated network prevails in determining the properties of oat flour at low level of milk constituents.

5.3.2 Hydration properties

The WAI and WSI of oat flour are presented in Table 5.2. The WAI is an indicator of the capacity of flour to hydrate and absorb water under certain thermal conditions, which can be correlated to give a desirable consistency and mouthfeel in a food system. The WAI values ranged from the highest in oat flour (4.82 g/g) to the lowest in oat flour substituted with 10% SMP (3.97 g/g). There was a significant difference observed in WAI capacity when oat flour was substituted with milk components at 5% and 10% substitution rate (P<0.05). However, there was no significant difference observed between each of the milk components at 5%

and 10%. Lupano, (2000) also measured a reduction in water absorption of starch-whey mixtures as whey protein concentrate was increased. The WSI is used to indicate leaching behaviour of starch degradation, and thus leached soluble molecules such as amylose and amylopectin contribute to water solubility index (Osundahunsi, Fagbemi, Kesselman, & Shimoni, 2003). In this study, water solubility index represents the presence of leached starch molecules, soluble sugars, protein etc. in the mixtures. WSI was significantly (P<0.05) increased in all the substituted samples in comparison with the control, which could be due to soluble nature of proteins and sugars present in milk components. However, SMP showed a trend of less WAI and WSI values in comparison with WPC and WLAC. Kumar et al. (2017) also represented the similar effects of milk components on oat starch.

Table 5.2 Hydration properties of oat flour containing milk ingredients

Samples	Water absorption index* (g/g)	Water solubility Index* (%)
Oat Flour	4.82±0.12^{a}	5.55±0.38^{f}
OF+WPC[2] 5%	4.33±0.09^{b}	10±0.88^{cd}
OF+WLAC[3] 5%	4.31±0.02^{b}	9.22±0.50^{de}
OF+SMP[1] 5%	4.28±0.13^{bc}	7.44±1.01^{e}
OF+WPC 10%	4.07±0.03^{cd}	12.44±0.69^{ab}
OF+WLAC 10%	4.01±0.02^{d}	13.66±0.66^{a}
OF+SMP 10%	3.97±0.02^{d}	11.33±0^{bc}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

5.3.3 Syneresis analysis

Syneresis refers to the expulsion of water from a gel structure, which is used to evaluate the ability of starch gels to withstand physical changes during cooling and thawing (Karim, 2000). Syneresis is related to retrogradation which is caused by molecular associations between leached starch molecules during cooling (Muadklay & Charoenrein, 2008). The syneresis of the whole oat flour with milk components was studied, and the results are shown in Table 5.3. Syneresis was significantly increased as the starch fraction was substituted with milk components. However, no significant difference was observed in syneresis among each milk component at 5% and 10% substitution of oat flour. Oat flour has a significant amount of fat (about 5%), which can be associated with starch molecules in the form of lipids and can influence syneresis (Autio & Eliasson, 2009). The phospholipids have a tendency to form complexes with leached amylose molecules during pasting and the resulting complex can hinder starch swelling and might increase starch retrogradation (Tester & Karkalas, 1996). This phenomenon could lead to high syneresis levels, this is also discussed in the increase in syneresis of oat starch gels when substituted with milk components, but in the case of oat flour, which is more complex system than oat starch, we were unable to observe the effect of individual milk components.

5.3.4 Large deformation viscoelastic properties

Large deformation studies can be correlated to stress-strain studies to reveal the textural properties of food products. Large strain deformation tests follow the disruption of food material and mimic the chewing in mouth or food processing. Moreover, for food gels and paste, these rheological tests give more insight to sensory characteristics related to product mouthfeel. As it can be depicted from the Table 5.3, all the penetration test responses showed a significant decrease ($p<0.05$) when oat flour was substituted with milk components.

However, if we compare the individual effects of the milk components on the oat flour gels, WPC and SMP had shown more similar and prominent effect on oat flour than WLAC. The hardness of the gels is attributed to the deformability of swollen starch particles and the amylose molecules present in the continuous network and presence of other flour constituents. This property can be affected by the presence of milk components. WPC and SMP showed more hardness at 5% and 10% substitution concentration in comparison with WLAC. Elasticity and brittleness also showed the same trend. This could be due to WLAC composition, which has a higher α- lactalbumin to β-lactoglobulin ratio than WPC, this leads to an inability to make strong gels because of α- lactalbumin resistance to denaturation and a tendency to phase separate (Wijayanti, Bansal, & Deeth, 2014b). Similar results were observed by in the chapter 4. As it is discussed in pasting section, WPC had the higher tendency to form whey aggregates and gel, which can contribute to strength of oat flour gel (Onwulata et al., 2003), while SMP consists of soluble milk minerals, lactose and casein micelles which could provide more junction points for intramolecular interactions of amylose chains and can lead to stiff gels (Gunaratne et al., 2007). Berski, Krystyjan, Zięć, Litwinek, and Gambuś, (2016) also reported an increase in gel hardness of oat flour gels in the presence of sugars.

Table 5.3 Penetration and syneresis properties of oat flour/milk ingredients mixtures

Samples	Hardness* (N)	Brittleness* (mm)	Elasticity* (N.s)	Adhesiveness* (N)	Syneresis* (%)
Oat Flour	0.141±0.014^{a}	5.46±1.90^{a}	0.029±0.001^{a}	-0.068±0.001^{d}	15.59±0.34^{c}
OF+WPC[2] 5%	0.122±0.013^{b}	4.49±0.51^{ab}	0.023±0.001^{b}	-0.062±0.002^{c}	17.36±0.06^{b}
OF+WLAC[3] 5%	0.095±0.006^{c}	4.09±0.13^{b}	0.019±0.001^{cd}	-0.060±0.003^{c}	17.44±0.11^{b}
OF+SMP[1] 5%	0.127 ±0.010^{ab}	5.20±0.39^{ab}	0.020±0.000^{c}	-0.056±0.002^{b}	17.092±0.58^{b}
OF+WPC 10%	0.093±0.004^{cd}	4.36±0.18^{ab}	0.016±0.000^{ef}	-0.050±0.000^{a}	18.74±0.44^{a}
OF+WLAC 10%	0.077±0.006^{d}	4.02±0.42^{b}	0.014±0.000^{f}	-0.053±0.003^{ab}	18.73±0.48^{a}
OF+SMP 10%	0.096±0.007^{c}	4.29±0.33^{ab}	0.018±0.001^{de}	-0.053±0.001^{ab}	18.73±0.29^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

[1] SMP-Skim Milk Powder, [2] WPC-Whey protein Concentrate, [3] WLAC-Whey Lactalbumin

In addition to the compositional differences between milk components, the effect of these components such as lipids, sugars, minerals and protein type affects the oat starch swelling behaviour by making complexes with leached starch molecules. These complexes can restrict starch swelling and limit the leaching of amylose from swollen granules, which can result in stiff gels (Jane et al., 1999; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005). Kumar et al. (2018) also described that SMP and WPC reduced the oat starch granule size, which could lead to more closely packed starch molecules leading to elastic gels (Srichuwong, Isono, Jiang, Mishima, & Hisamatsu, 2012).

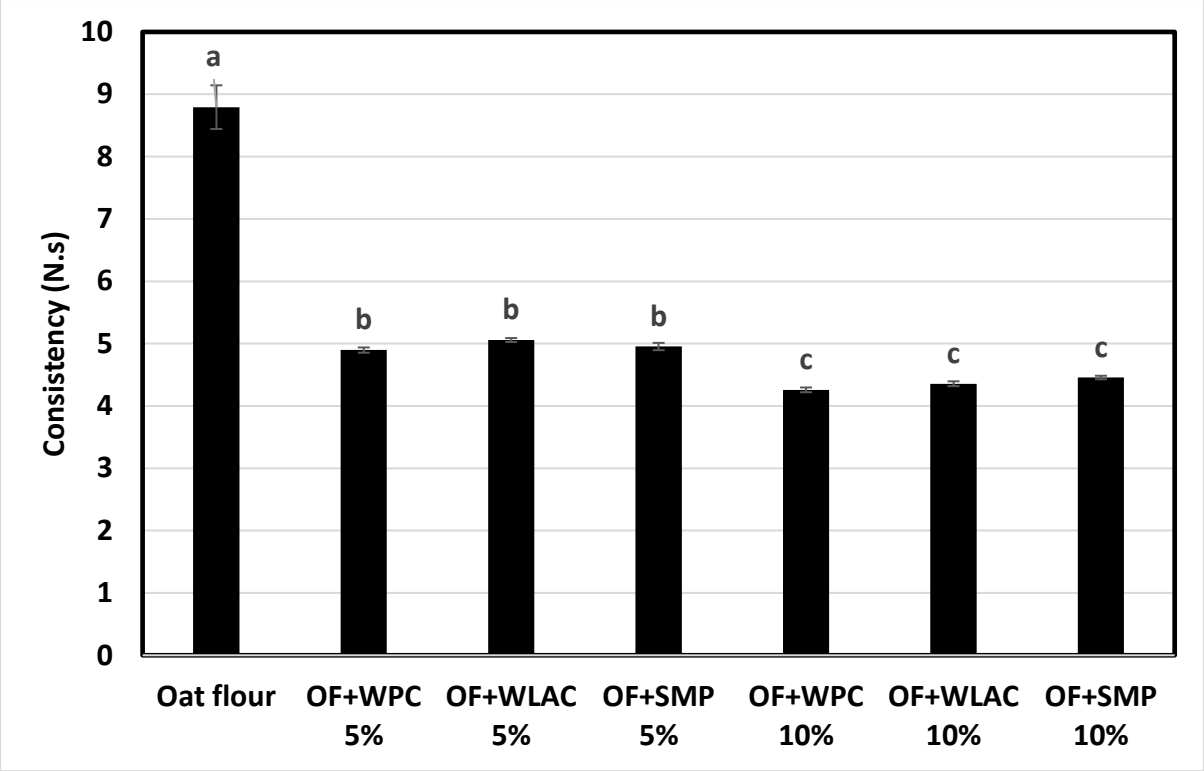


Figure 5-1 Consistency of oat flour paste containing milk components

Large deformation mechanical tests, especially back extrusion analysis of oat flour paste provided information that is more related to sensory attributes of a paste including mouthfeel. Oat flour consistency was measured, and the data was taken as representative of the extrusion force profiles in relation to time. The area under the curve or extrusion work was used as a measure of system consistency, which is commonly used in dairy products such as yoghurt (Angioloni & Collar, 2009). As can be observed from Fig 5-1 and 5-2, the substitution of oat flour with milk components led to a significant decrease ($p<0.05$) in extrusion properties. The consistency of oat flour decreased significantly with substitution by milk components, a significant drop was observed in the consistency of oat flour with 5% substitution of the oat flour fraction. However, the consistency also significantly reduced with 10% substitution, although it was not as large as the reduction between oat flour and 5% substitution. There was no significant difference found at each substitution rate among milk components.

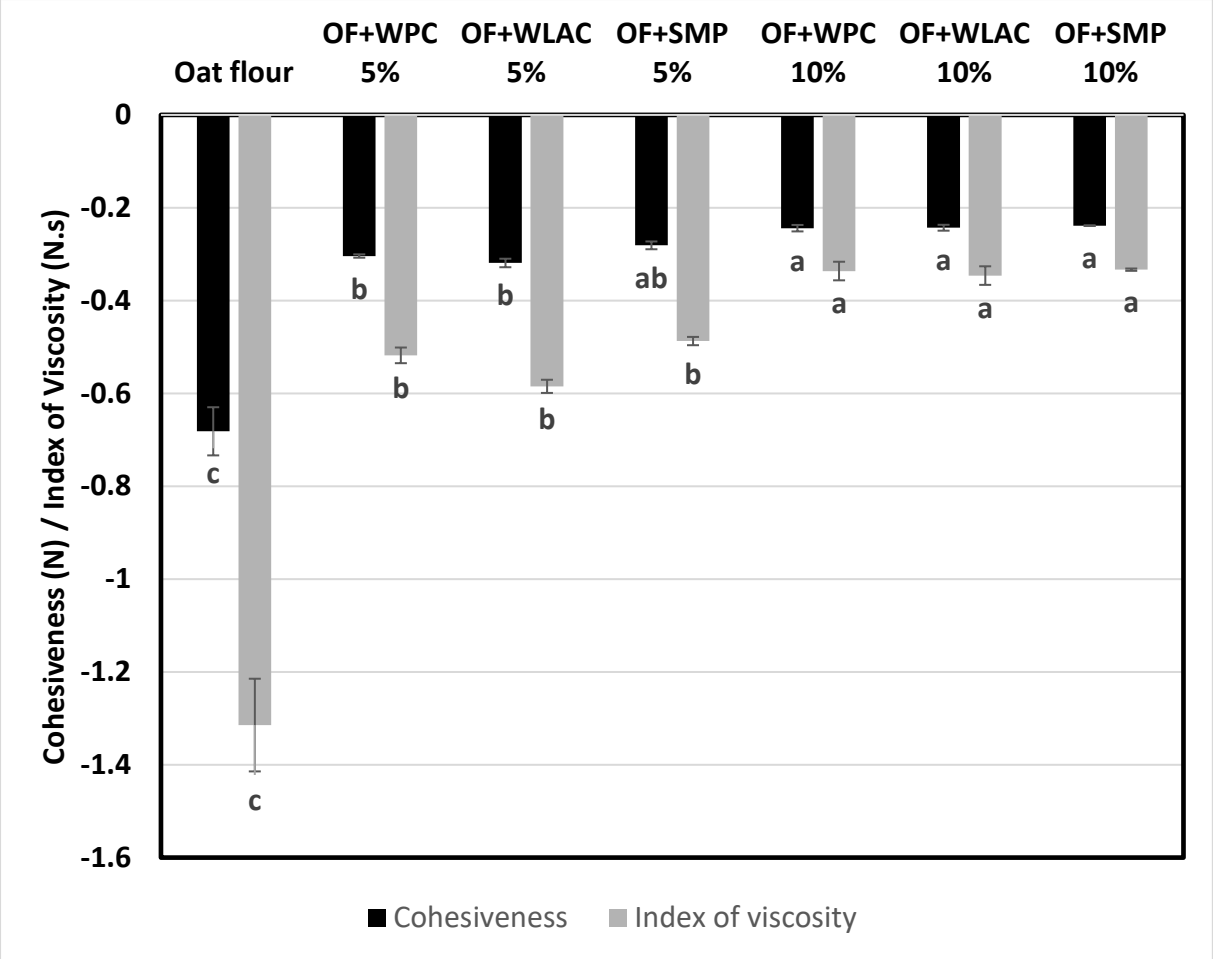


Figure 5-2 Cohesiveness and index of the viscosity of oat flour paste containing milk ingredients

Cohesiveness is the maximum negative force recorded during force-time profile. Cohesiveness was attributed to the cohesive force between intramolecular particles, by which the elements of materials are held together. It is related to the internal stickiness of a product; cohesiveness was reduced because of substitution of the starch fraction. Cohesiveness is attributed to the breakdown of starch molecules during heating and stirring; this result can give an insight into developing a food product where non-stickiness is required. The area under the peak negative force of a force-time graph was calculated as an index of viscosity (Figure 5-2), at 5% and 10% flour substitution with milk components resulted in a decrease in the viscosity index. The index of viscosity could give indirect measurement of flow behaviour of oat flour paste, which

can be used in determining the process conditions such as flowability. Notably, there was no significant difference observed among milk components.

5.4 Conclusion

The substitution of oat flour with milk ingredients resulted in a decrease in oat flour viscosity when gelatinized. Pasting properties suggested that WPC and WLAC have more effect on oat flour viscosity than SMP, while increased pasting temperature by SMP indicated resistance to swelling of starch molecules present in oat flour. The addition of milk ingredients increased the syneresis, irrespective of the type of milk components added. Large deformation studies indicated that SMP and WPC could result in more elastic and stiff gels, while consistency and cohesiveness of flour paste were also reduced by substitution of milk components. There was a sudden change in oat flour paste properties at 5% in comparison with control, but the very small difference was observed from 5% to 10%. Finally, this study reveals the complex behaviour of oat flour in the presence of milk ingredients, although further investigation is required to fully understand the interactions between oat flour components and milk components. The trends obtained from this study could predict the usage of oat flour with milk components in the development of novel food products, textures and overall acceptability.

Chapter 6

6 Influence of Whey proteins on thermal, pasting and structural characteristics of oat starch

6.1 Introduction

In the previous chapter, it was observed that readily available common milk protein ingredients had an effect on oat starch and flour, when oat starch was substituted with these protein ingredients. To understand the effect of milk protein on oat starch, pure isolate of whey proteins in the form of WPI were selected to determine its interactive effects with oat starch .

Starch is a very common ingredient used in industry for controlling food texture due to its gelling and stabilizing properties. When starch is heated in water above to a certain temperature (which is normally the gelatinization temperature), it loses crystallinity by absorbing water and swells to a degree, where amylose and amylopectin leach out and provide viscosity. Oat starch exhibits typical properties including small granule size, a well-developed granule surface, and high lipid content (Hoover et al., 2003; Mirmoghtadaie et al., 2009). Whey protein isolates (WPI) have characteristics such as, gelling due to aggregation, thickening and water-binding capacity. WPI (>90%) mainly consists of two subfractions: β-lactoglobulin (>70% of total protein) and α-lactalbumin (15–20%) of total protein. The gelling behaviour of WPI is due to denaturation and unfolding of protein chains and aggregation of proteins into a three dimensional network through hydrophobic or hydrogen bonding (Fitzsimons et al., 2008). Fitzsimons et al., (2008) suggested two overlapping endothermic transition peaks for WPI and starch, the resulting peaks represented a typical gelatinisation endotherm peak (63 ^{o}C) and β-lactoglobulin endotherm peak (74 ^{o}C). Furthermore, these unique properties of WPI can affect starch native properties and could have a effect on

resulting food textures.

There are no studies on oat starch interaction with whey proteins and in systems in which whey proteins and oat starch co-exist, it is important to understand the mechanisms, interactions and synergistic effects that can provide maximum benefit to oat-milk based food products. Hence, the aim of this study was to investigate the effect of Whey proteins on oat starch thermal, pasting and structural properties.

6.2 Material and Methods

6.2.1 Materials

This section is described in 3.1.

6.2.2 Sample preparation

The mixtures with oat starch and whey protein isolate (WPI) were prepared as 1:0 (0% WPI), 1:0.25 (25% WPI), 1:0.5 (50% WPI), 1:0.75 (75% WPI) and 1:1 (100% WPI) and starch concentration was kept constant 10 % (w/w) for all the experiments.

6.2.3 Rheometer Pasting properties

This section is described in 3.2.13.

6.2.4 DSC analysis

This section is described in 3.2.14

6.2.5 X-ray diffraction (XRD) analysis

This section is described in 3.2.15

6.2.6 Fourier transform infrared (FTIR) spectroscopy

This section is described in 3.2.16

6.2.7 Gel Scanning electron microscopy (SEM)

This section is described in 3.2.17

6.2.8 Statistical analysis

This section is described in 3.2.19

6.3 Results and Discussion

6.3.1 Pasting properties

The different pasting responses for the addition of WPI to oat starch are presented in Table 6.1. These results clearly show that WPI affected the oat starch pasting properties markedly, Peak viscosity decreased significantly ($p < 0.05$) when low (25% and 50%) WPI concentrations were added and increased on the addition of higher (75% and 100%) WPI concentrations. The peak viscosity is related to starch swelling, and the extent of starch swelling at continuous shear and heat (Sopade et al., 2006). The final viscosity followed the same trend; particularly when WPI 100% was added.

The oat starch/WPI mixtures showed that at low concentration of WPI, peak viscosity was decreased, in this case the aggregation behaviour of WPI was minimal compared to high WPI concentration (75% and 100%) which contributed to increased peak and final viscosities. During the denaturation of whey proteins, the free thiol group has been shown to be exposed and reacts with sulfhydryl groups of other proteins to form dimers, trimers and tetramers (Walstra, P., Wouters, Jan T., & Geurts 2006). At low concentrations, these aggregates may remain small and does not contribute to overall viscosity, whilst at high concentrations, they can form a gel and as the concentration of biopolymers present in the system are increased. This can result in an increased local concentration of starch molecules which lead to higher viscosities.

Noisuwan et al. (2008) also reported the same observations that whey proteins increased the

peak and final viscosities of rice starch. Paste stability is expressed by either breakdown viscosity or stability ratio in a pasting profile. Low breakdown viscosity, high stability ratio is often related to high shear resistance and low hydration of starch system (Shafie et al., 2016). The stability ratio was increased when WPI added to the oat starch system, which indicated less starch swelling and less breakdown of starch granules. Setback ratio and setback viscosity showed the retrogradation behaviour of gelatinised starch gels when cooled to 50 °C. Setback ratio was decreased significantly ($p < 0.05$) in comparison with the control when whey proteins were added to the starch system and showed prominent effect at 50% WPI concentration. It can be observed from Figure 6.1 that the formation of WPI aggregates can hinder re-association of amylose molecules, hence resulting into reduced setback viscosity and setback ratio. The relative breakdown indicated the extent of disruption of starch granules in the presence of WPI, which was decreased as WPI added in the system. Finally, WPI affected the pasting behaviour of oat starch, which is due to competition for water between the starch molecules and whey proteins, the other reason is ability of whey proteins to form whey aggregates which can interact with starch molecules and can form gels leading to increase in viscosity.

6.3.2 Thermal properties

The DSC parameters (T_o= onset temperature of gelatinization endotherm; T_p = peak temperature; T_c = conclusion; $\Delta T = T_c - T_o$ and ΔH= enthalpy change) of oat starch alone and oat starch/WPI mixtures are shown in Figure 6-2 and Table 6-2. Starch is made up of semi-crystalline solid and during gelatinization this ordered structure is disrupted and melted; this results in uptake of water by amorphous and crystalline regions of starch and swelling of starch (Autio & Eliasson, 2009).

Table 6.1 Pasting properties of oat starch/WPI mixtures

Samples	Peak Viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final (cP)	Setback (cP)	Stability Ratio	SetBack Ratio	Relative Breakdown
Oat Starch	3218.4±20.8^{c}	1951.82±12.65^{e}	1266.6±16.7^{b}	4948.3±18.6^{c}	2990.47±6.07^{b}	0.608±0.000^{e}	2.52±0.006^{a}	0.421±0.001^{a}
WPI 25%	2859.78±13.0^{d}	2150.97±8.37^{d}	708.8±21.4^{c}	4296.18±12.83^{d}	2145.2±21.2^{d}	0.752±0.006^{c}	1.99±0.013^{c}	0.330±0.006^{b}
WPI 50%	2707.68±9.03^{e}	2268.4±14.2^{c}	439.31±5.20^{e}	4272.14±3.92^{d}	2003.77±10.31^{e}	0.837±0.002^{a}	1.88±0.010^{d}	0.219±0.001^{d}
WPI 75%	3307.48±2.72^{b}	2664.45±13.29^{b}	643.00±16.0^{d}	5328.22±4.73^{b}	2663.77±8.56^{c}	0.805±0.004^{b}	1.99±0.008^{c}	0.241±0.005^{c}
WPI 100%	5514.32±4.86^{a}	3811.82±13.46^{a}	1702.50±8.60^{a}	9211.13±9.04^{a}	5399.31±4.42^{a}	0.691±0.001^{d}	2.41±0.006^{b}	0.315±0.001^{b}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at $p<0.05$**

Table 6.2 Thermal analysis parameters of oat starch and oat starch/WPI mixtures

Samples	T_o^* (◦C)	T_p^* (◦C)	T_c* (◦C)	ΔT (◦C)	ΔH (j/g)
Oat Starch	56.350±0.070^d	61.200±0.283^d	67.000±0.283^c	10.650±0.354^a	12.040±0.085^a
WPI 25%	57.425±0.035^c	62.165±0.063^c	66.515±0.120^c	9.090±0.156^a	13.48±0.439^b
WPI 50%	57.655±0.049^b	63.490±0.042^b	68.015±0.120^b	10.360±0.070^a	15.8±0.156^c
WPI 75%	57.990±0.056^a	63.995±0.148^b	68.160±0.042^b	10.170±0.014^a	18.31±0.185^d
WPI 100%	58.070±0.028^a	64.815±0.134^a	68.895±0.007^a	10.825±0.021^b	22.44±0.148^e

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

In systems with excess water this happens over a very narrow temperature range and therefore results in a single endothermic transition (Biliaderis, Maurice, & Vose, 1980; Donovan, 1979).

The swelling of starch and leaching of starch molecules such as amylose and amylopectin results in a change of rheology of the system; which is crucial for sensory and textural attributes of food. In the present study, the oat starch was evaluated when in the presence of excess water, in this case with the DSC a single endothermic transition occurred as expected. The DSC results of oat starch was found to be in agreement with previous results (Hoover et al., 2003; Šubarić et al., 2011).

Differential scanning calorimetry (DSC) heating traces showed two overlapping endothermic transition peaks for WPI and starch mixtures at all concentrations of WPI, the resulting peaks represented a typical starch gelatinisation endotherm peak (61~64 °C) and β-lactoglobulin endotherm peak (74 °C) (Fig. 6.2). Studies with DSC revealed that a mixture of WPI and starch enhanced thermal stability of mixture but did not support a direct association of both biopolymers. Fitzsimons, Mulvihill, and Morris (2008) and N. Yang, Ashton, and Kasapis (2015) also reported the two peaks, the first peak was attributed to starch gelatinisation while second peak observed was attributed to the protein denaturation.

As can be observed from Table 6.2 and Figure 6.2, addition of WPI significantly ($p < 0.05$) increased the onset, peak and conclusion temperature of the endothermic starch transition in comparison with oat starch only. However, the increase in peak and conclusion temperature of the 50% and 75% starch/WPI mixtures were not significantly different from each other. The DSC endotherm revealed a remarkable increase in enthalpy (ΔH) of WPI/starch mixtures in comparison with oat starch alone, which is in increasing order of WPI concentration and highest at 100% WPI of oat starch addition.

The higher transition temperature (ΔT) was also noted in 100% WPI/starch mixture in comparison with other samples, which might be due to higher amount of WPI in the mixture and supported of increased enthalpy. The increase in enthalpy (ΔH) could be due to uptake of water by whey proteins and more heat needed for protein denaturation of second endothermic peak, which resulted in overall increase in enthalpy for the system. Similar results were reported by Noisuwan et al. (2008) and Fitzsimons, Mulvihill, and Morris (2008), where they found two endothermic peaks when whey proteins are added in rice and cassava starch respectively.

Lupano and González (1999) reported the WPC effect on cassava starch at pH 4.2, where cassava starch gelatinization temperature was increased on the addition of WPC, this behaviour was explained by the fact that the water absorption by WPC led to reduced water availability for swelling and gelatinization process of starch. Yang, Ashton, and Kasapis (2015) studied the chitosan effect on WPI/wheat starch composites and reported the same effect that increase in gelatinization temperature of starch due to hydration of whey proteins and less availability of water for starch swelling.

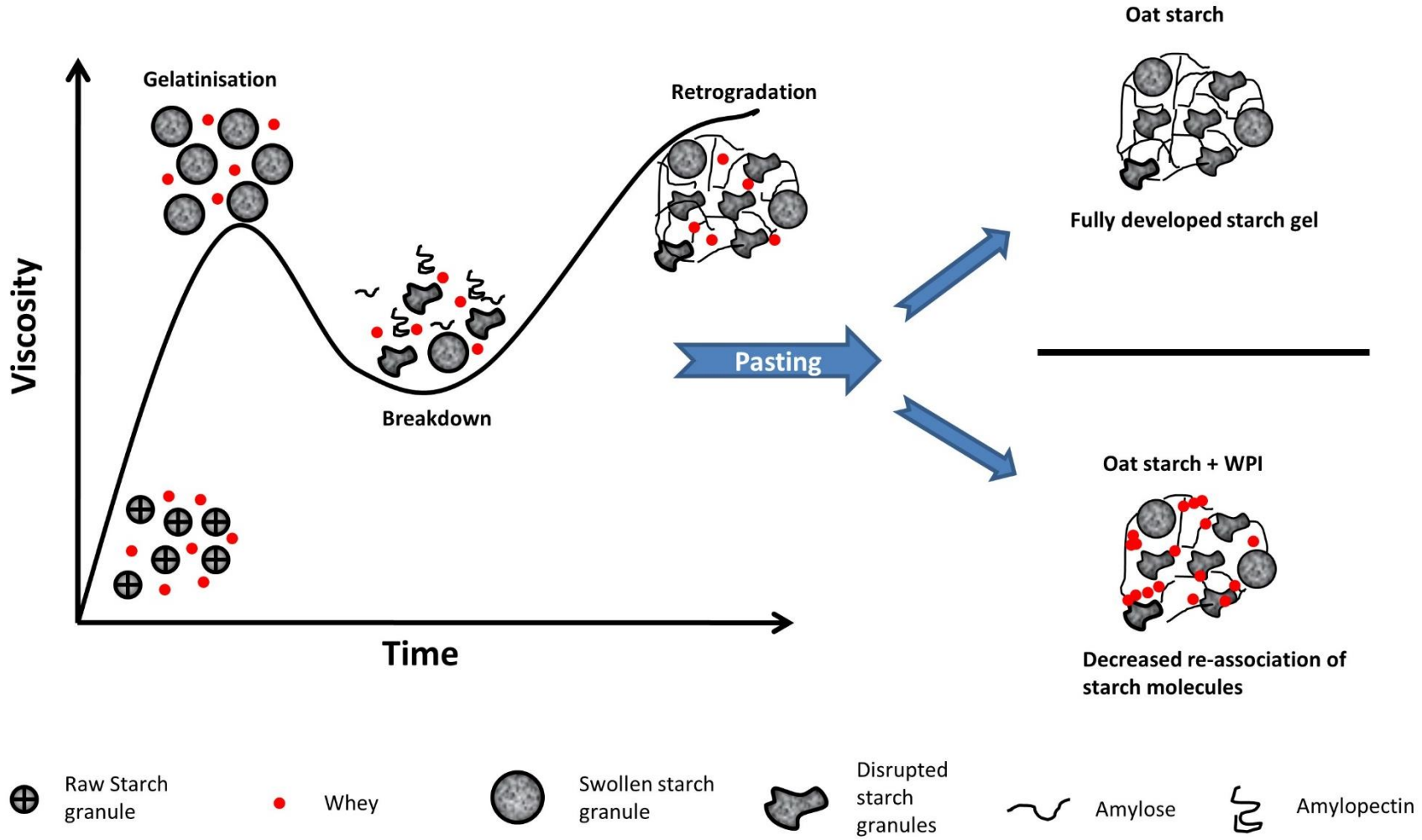


Figure 6-1 Schematic representation of WPI effect on pasting properties of oat starch

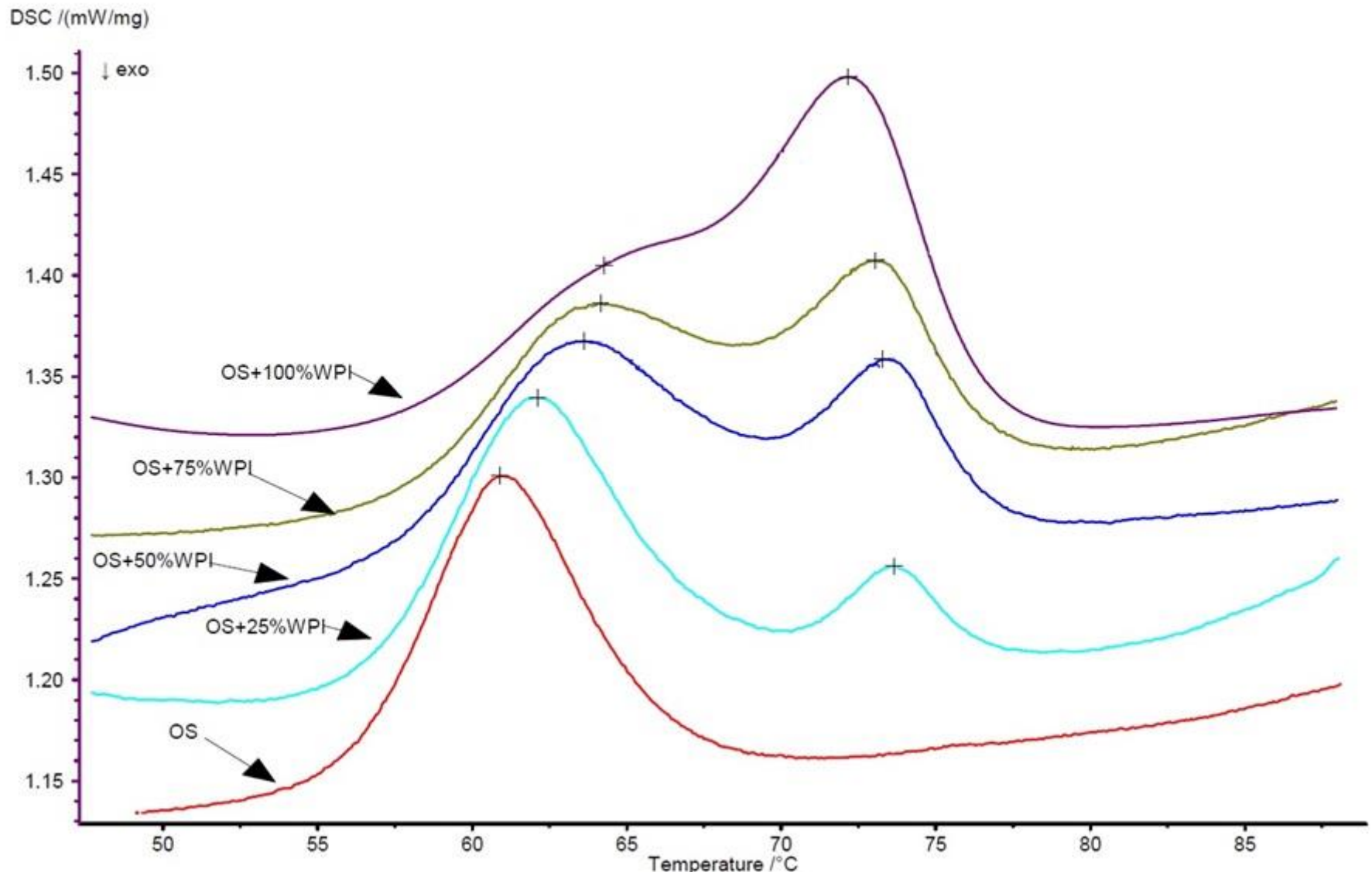


Figure 6-2 DSC thermograms of oat starch and oat starch/WPI mixtures

6.3.3 FTIR analysis

FTIR spectroscopy was used to examine oat starch/WPI gels; the vibrational frequencies between atomic bonds in mixtures are recorded as absorption peaks in Figure 6-3 and 6-4. Starch granules are made up from two molecules, amylose and amylopectin. These glucose polymers are packed into a complex semi-crystalline granular arrangement and when starch is heated in excess of water, water interacts with amorphous regions leading to melting of crystallites, swelling of starch molecules and loss of ordered structure (Perry & Donald, 2002). FTIR can be used to measure the structural changes at a molecular level by disruption of short range double helical crystalline order in starch during gelatinization process (Bogracheva, Meares, & Hedley, 2006), which might be affected by the addition of whey proteins in our study. No new bands were observed by the addition of WPI to starch, which shows the absence of covalent bonding between the two biopolymers. Hydrated starch gels mixtures showed pronounced peaks at 3000-3700 cm^{-1}, 2000-2250 cm^{-1}, 1500-1750 cm^{-1} and 1000-1200 cm^{-1}, while Starch/WPI mixtures showed some additional bands at amide I and amide II region. The starch typically shows bands at 2900–3000 cm^{-1} (C-H stretching), 1100–1150 cm^{-1} (C- O, C-C and C-O-H stretching) and 1100–900 cm^{-1} (C-O-H bending). It is not possible to assign bands in the spectra of starch unambiguously, as the absorbance bands overlapped and were poorly resolved (Van Soest & Vliegenthart, 1997). The bands in the region 1100–900 cm^{-1} have been shown to be sensitive to changes in starch structure. As it can be shown from Figure 6-4, The IR bands intensity at ~1024 cm^{-1} and ~1080 cm^{-1} decreased the addition of WPI in the system except 25% WPI inclusion, where it showed an increment. The IR bands at ~1022-1024 cm^{-1} have been suggested to be associated with the amorphous structure of starch (Zeng, Ma, Kong, Gao, & Yu, 2015). The absorbance at 1024 cm^{-1} is commonly associated with freshly prepared starch gel and indicates gelatinisation.The absorbance is assigned to

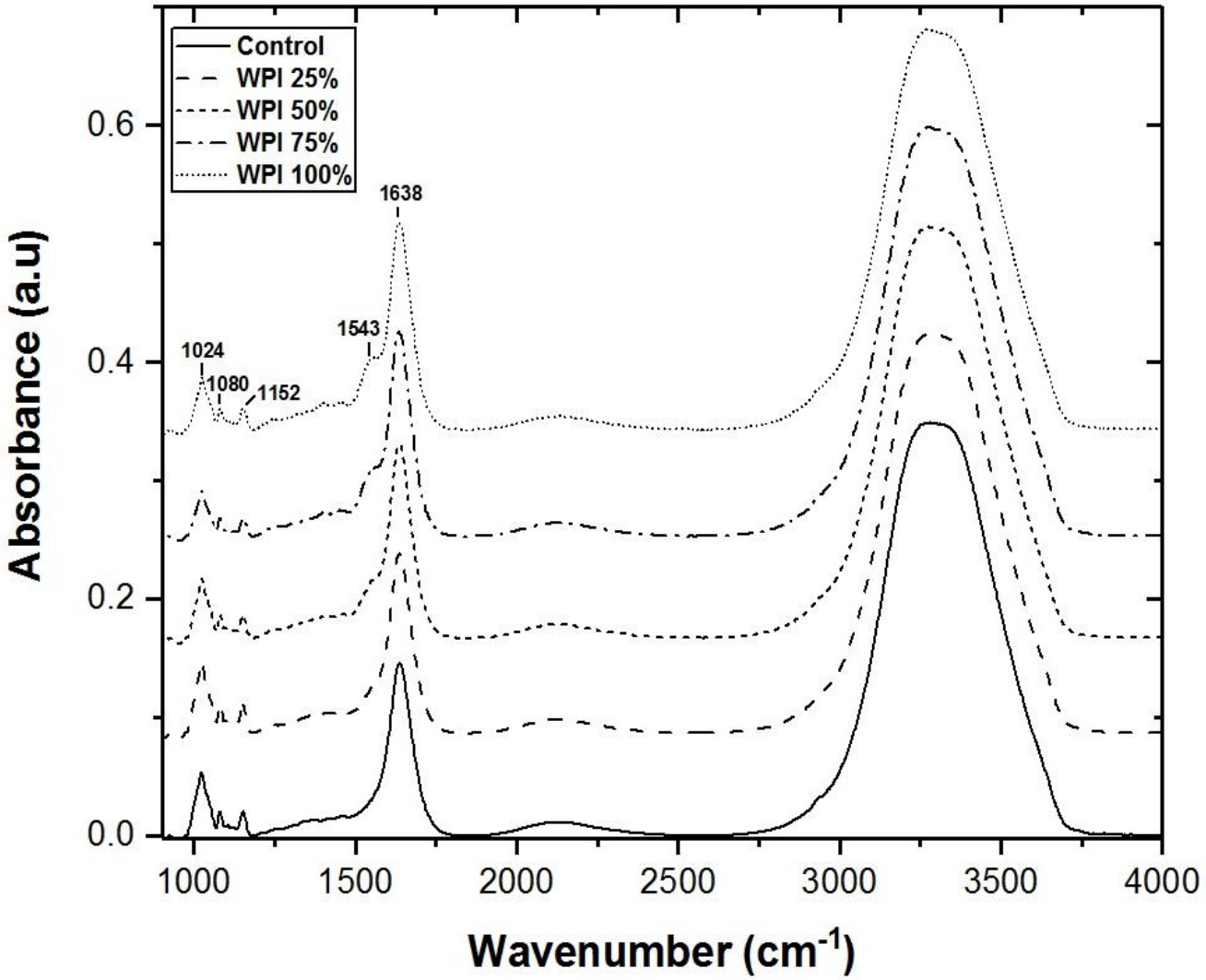


Figure 6-3 FTIR spectra of oat starch with WPI

C-O-H group vibrations, the increase in intensity of band at 1024 cm^{-1} shows a decrease in crystallinity (van Soest et al. 1995). The FTIR spectra illustrated that addition of WPI decreased the absorbance intensity at 1024 cm^{-1}, which indicated the increase in ordered structure or more uniform order of amorphous phase. This result indicates indirectly that whey proteins affect starch gelatinization properties and hinder the process of gelatinization, the same behaviour is also observed in DSC studies where WPI increased the peak temperature and enthalpy. The absorbance at 1080 cm^{-1} is also assigned to stretching of C-O,C-C and O-H bonds and an alternative indicator of ordered structure, the band at 1152 cm^{-1} also suggested the amorphous order of starch (Rubens, Snauwaert, Heremans, & Stute, 1999; Warren, Gidley, & Flanagan, 2016). Similar results were reported by Rivera-Corona et al., (2014), where sorghum starch properties were affected by the addition of cactus mucilage. The amide I band region which is located between 1600-1700 cm^{-1} and centred at 1638 cm^{-1}, showed an incremental increase with the addition of WPI. The IR band at 1638 cm^{-1} was associated with β-sheets structure of proteins and represented the intermolecular β-sheets formation upon protein denaturation and aggregation by heat (Kong & Yu, 2007). This result indicates that intermolecular β-sheets were formed, and the β-sheets have higher degree of intermolecular hydrogen bonding, which need higher energy to break the bonds and can result in strong gel (Guerrero, Kerry, & De La Caba, 2014). The signal at 1547 cm^{-1} corresponded to the amide II region contributing to peptide bond group vibrations. The vibration absorbance bands at 1406 cm^{-1} and 1452 cm^{-1} corresponds to $-CH_2$ moieties of amino acid side chains (Barth, 2000). Finally, it can be concluded from the FTIR studies that WPI addition affected the short range crystalline ordered structure of oat starch and the internal structure was more inclined to more ordered form, resulting in restricted starch swelling and gelatinization process and FTIR studies supported the DSC results.

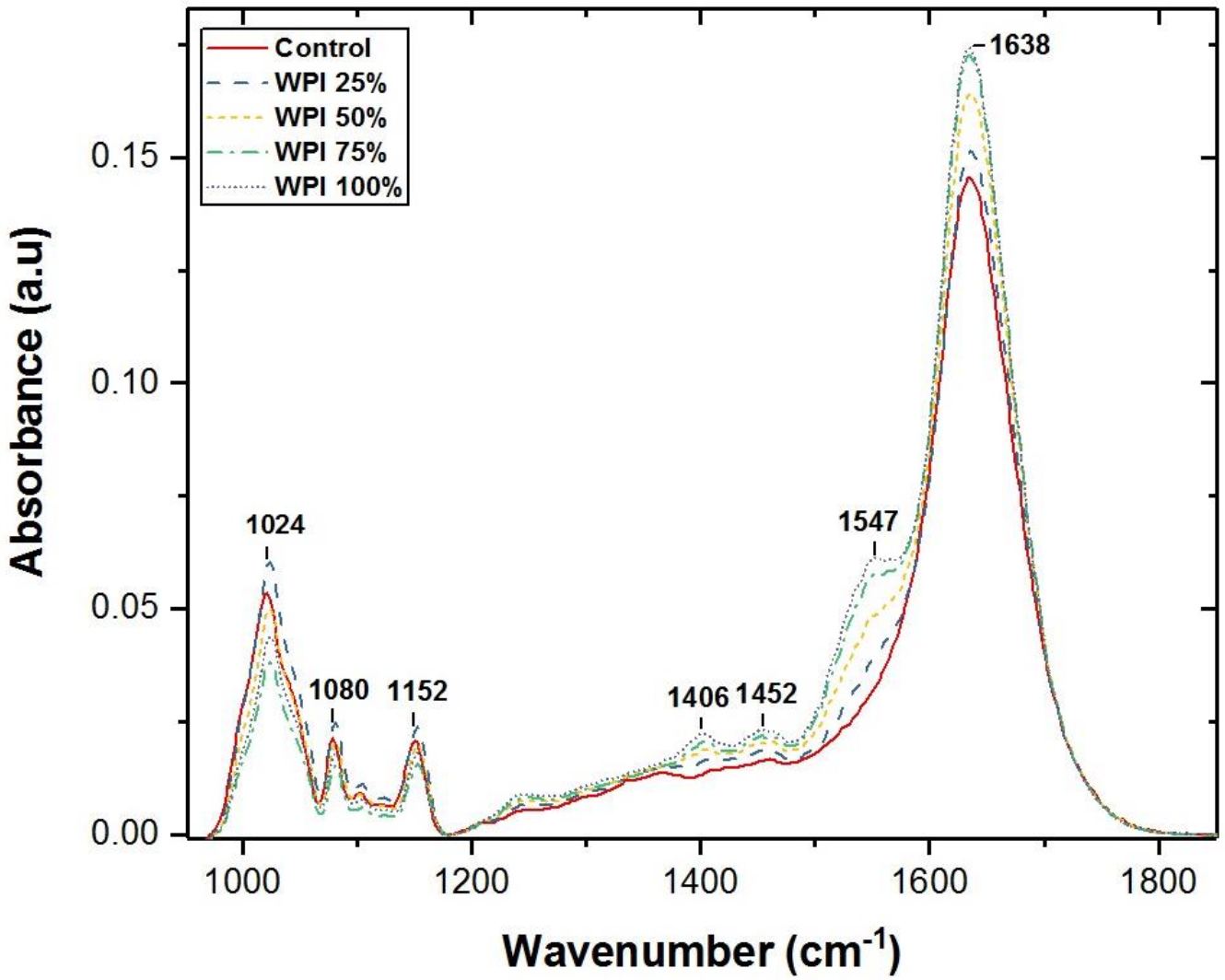


Figure 6-4 FTIR spectra showing amide I and amide II region with starch sensitive bands (1000-1200)

6.3.4 XRD analysis

X-ray diffraction (XRD) method is a way to assess and quantify the long-range crystalline order of starch, as starch is a semi crystalline material, XRD uses incoherent scattering from crystalline regions to assess the molecular ordering of the starch. The crystalline and amorphous region varies from 10% to 50% and depends upon botanical origin of starch (Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008). This pattern can be altered by heat treatment during gelatinization, where the starch is heated in the presence of excess water at high temperature >60 ◦C.

In this process hydration of amorphous regions occurs leading to destabilising and melting of crystalline regions (Bogracheva et al., 2006; Perry & Donald, 2002). This process can be affected by the presence of whey proteins in the system, as depicted by DSC and FTIR analysis. The two-phase model is used, where an amorphous region is subtracted from crystalline pattern, and the ratio of two phases gives relative long-range crystallinity (Sterling & Pangborn, 1960). The XRD pattern of the native oat starch is shown in Figure 6-5.

Oat starch showed peaks at 15^{o}, 17^{o}, 18^{o}, 20^{o}, 23^{o}, 26^{o} and 30^{o}, which are characteristic of a A-type pattern. Previous studies have also shown the same XRD pattern of oat starch (Autio & Eliasson, 2009; Dar et al., 2018; Shah et al., 2016a; Zhou et al., 1998). The relative degree of crystallinity of oat starch was 22.23%, which is close to the oat starch crystallinity studied by (Binqiang, Chao, Lan, & Bijun, 2016). It can be depicted from Figure 6-6 that the oat starch A-type diffraction pattern is shifted from crystalline ordered structure to more random amorphous state by the action of high temperature and shearing process, which is a characteristic of gelatinization during pasting. The broad peaks at 20^{o} and 35^{o} represented the amorphous region of the starch. Similar XRD pattern is represented by a study of González-

Gutiérrez et al. (2011), examining albumen/starch composites.

The addition of WPI in the oat starch system did not modify the diffraction pattern of the system but resulted an increase in the diffraction pattern intensity. This increment was found to be relative to the increase in WPI concentration. This suggested that WPI addition resulted in a more ordered structure of oat starch/WPI mixtures. The relative crystallinity (RC) of oat starch mixtures increased from 22.2% to 26.7% in 100% WPI/starch mixture (Figure 6-6). The change could be attributed to the WPI influence on starch gelatinization properties. The increase in the RC was in agreement with the increase in enthalpy, peak temperature and decreased bands at 1024 cm^{-1} and 1080 cm^{-1} studied by DSC and FTIR as discussed earlier in section 6.3.2 and 6.3.3.

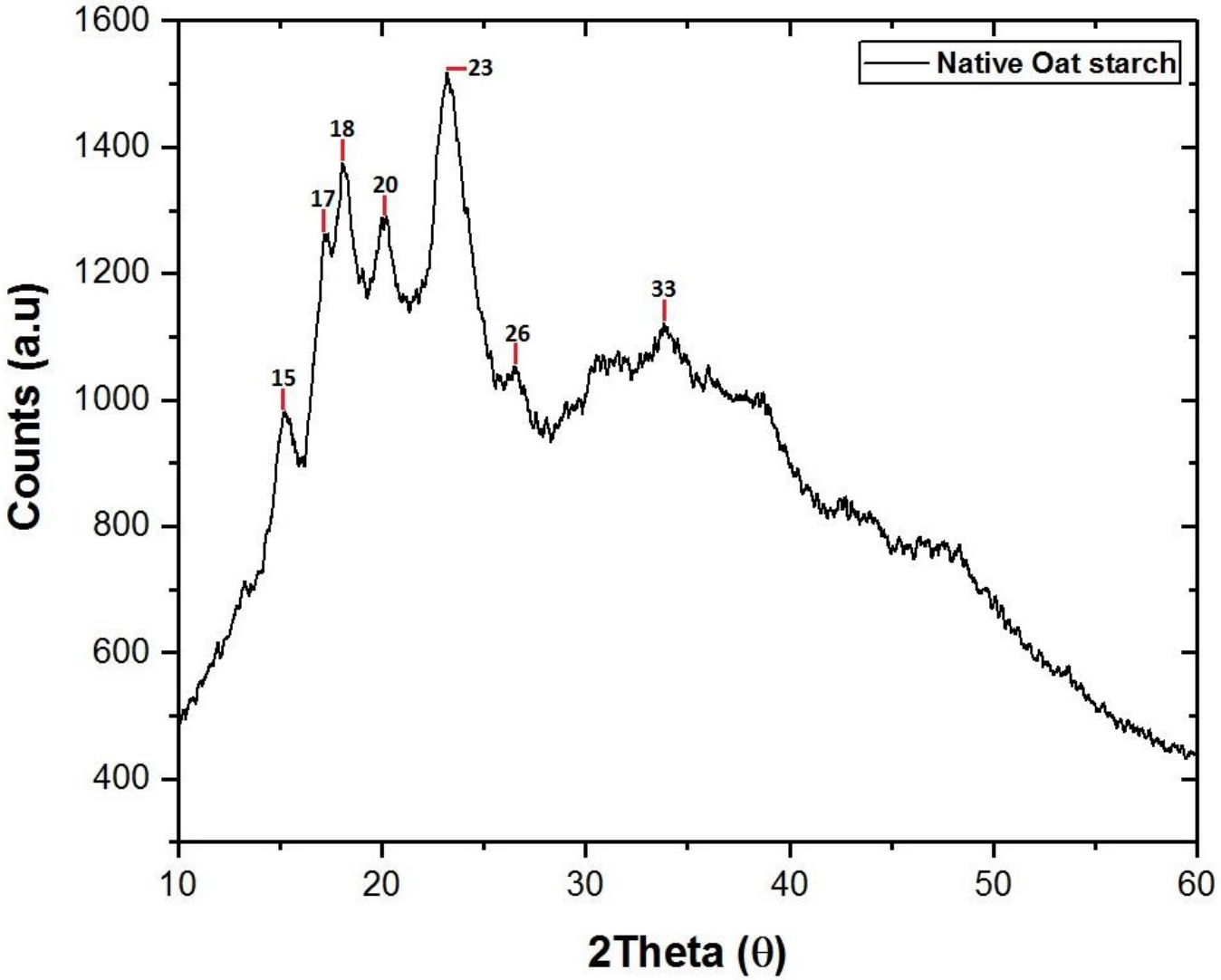


Figure 6-5 XRD diffraction pattern of oat starch

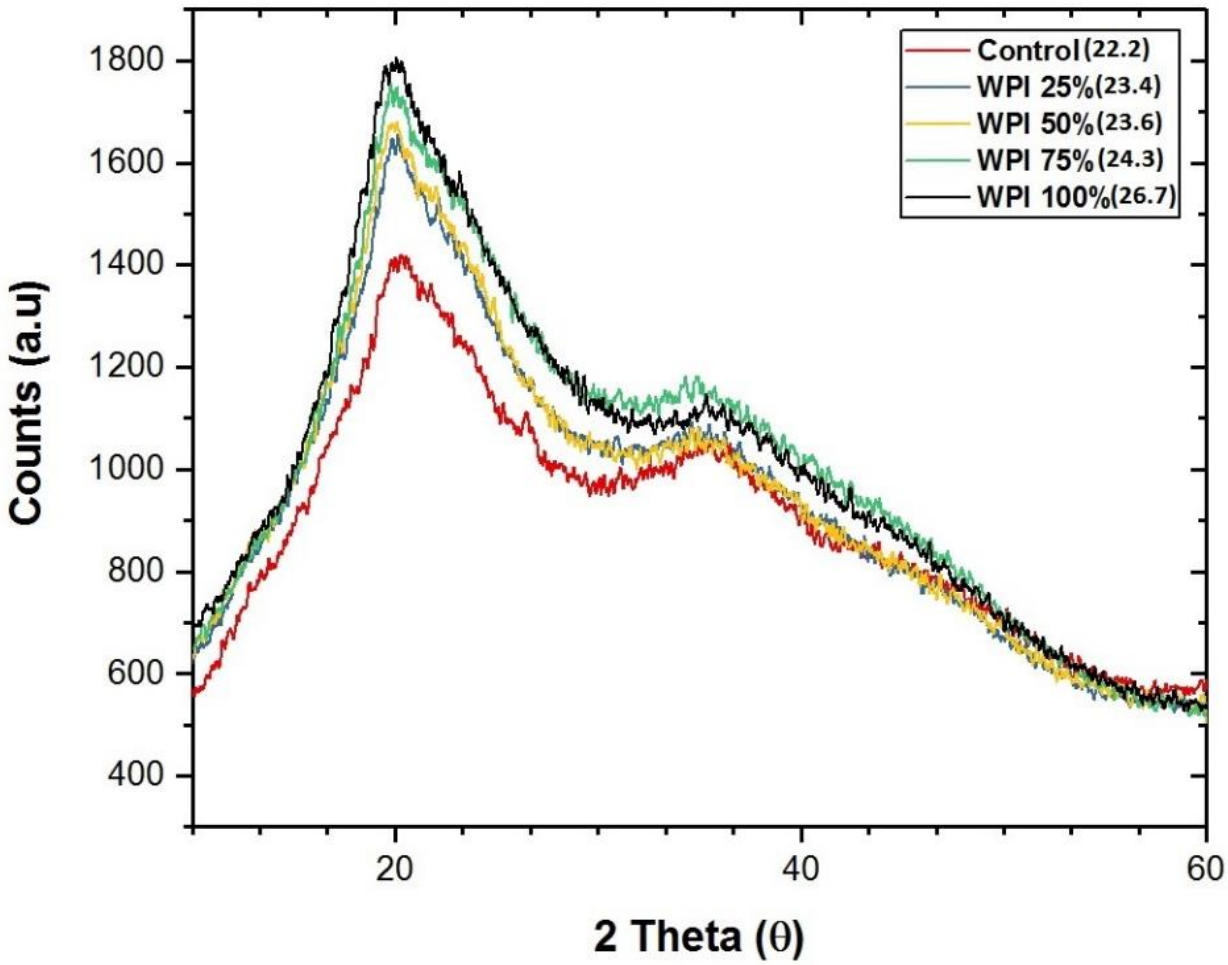


Figure 6-6 XRD diffractogram of oat starch/WPI freeze-dried gels

6.3.5 Scanning electron microscopy

The SEM micrographs of freeze-dried oat starch-WPI gels at different protein concentrations are presented in Figure 6-7. The micrographs show a typical starch/protein composite gel internal structure, and are similar to micrographs reported by Li, Yeh, and Fan, (2007) and (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014) for soy protein/corn starch and lentil protein/lentil starch gels respectively. All the oat starch/WPI gels showed a well-developed three-dimensional gel network with a honey comb porous microstructure. No starch granules or remnants were observed in the micrographs, which suggest a complete pasting process or starch gel formation. The honey comb porous structure, which appeared as interconnected

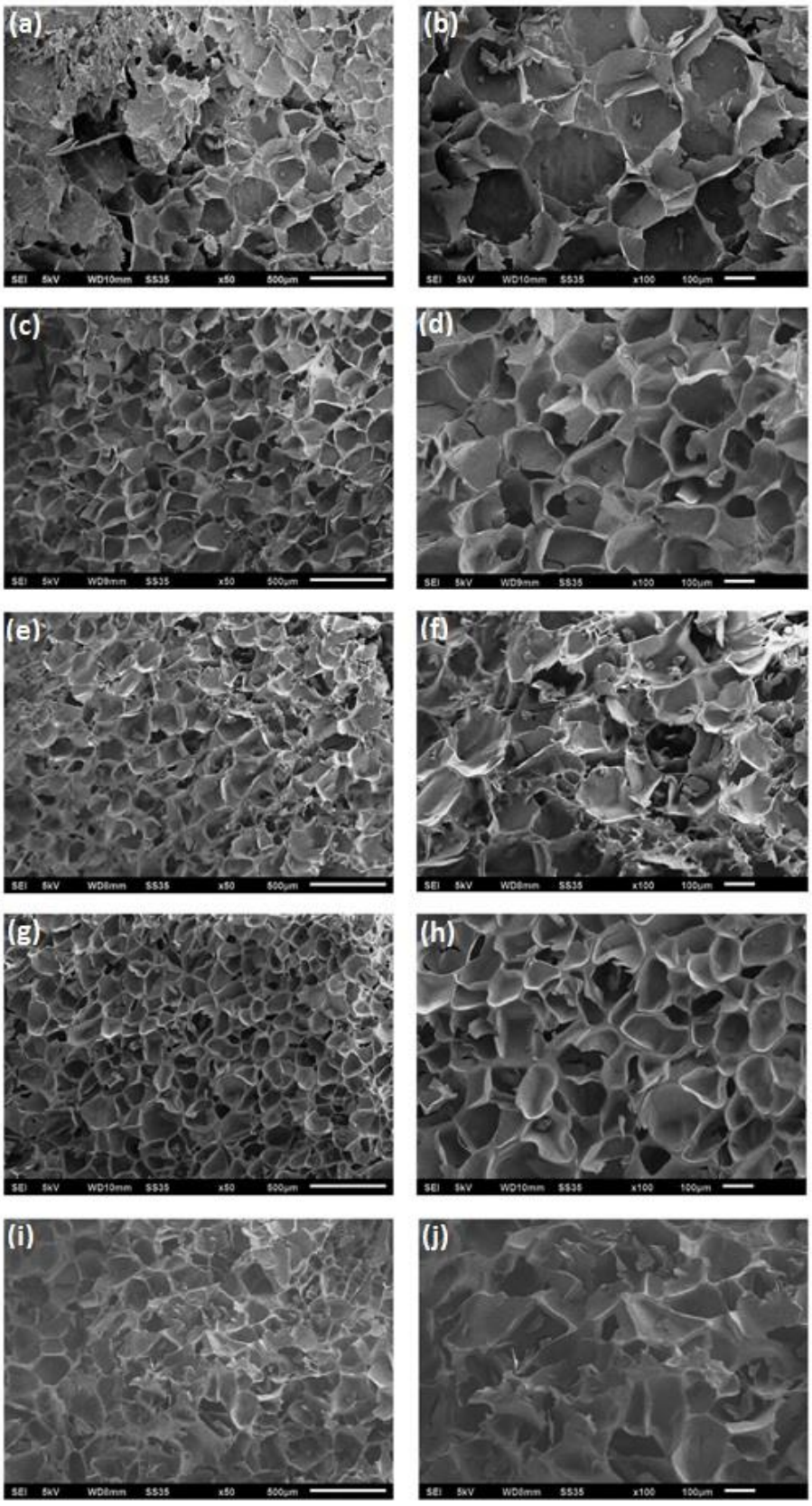


Figure 6-7 SEM micrographs of oat starch/WPI freeze dried gels indicating oat starch (a-b), 25% WPI/starch (c-d), 50% WPI/starch (e-f), 75% WPI/starch (g-h) and 100% WPI/starch (i-j) mixtures

tunnels, is responsible for gel elasticity and the degree of density of these voids indicates the strength of the gel (Joshi et al., 2014). The oat starch gel structure appeared to have a large pore size and a shallow pore structure, which is an indication of strong starch gel formation (Figure 6-7, a-b). The microstructure of oat starch/WPI gels is presented in Figure 6-7(c-j), indicating 25% WPI/starch (c-d), 50% WPI/starch (e-f), 75% WPI/starch (g-h) and 100% WPI/starch (i-j) mixtures. As can be seen from the Figure 6-7(c-j), upon incorporation of WPI, the structure of WPI/starch gels became denser and there was a reduction in the pore size of the structure. The oat starch/WPI gels containing higher protein fractions showed well developed and denser pores onto which a WPI protein mass layer was dispersed, particularly at 75% WPI/starch mixture (Figure 6-7, g-h). The network structure of the starch/WPI gels was found to be dependent on the starch/WPI ratio. At high WPI/starch concentration such as 100% WPI/starch, a denser and more flaky appearance of the internal structure was observed, this might be due to the gel formation by both the polymers starch and WPI. Similar microstructures were observed by Dumay et al. (1999) on β-lactoglobulin/polysaccharides gels.

6.4 Conclusion

The addition of the different concentration of WPI affected the thermal, pasting and structural properties of oat starch. Peak viscosity and final viscosity of oat starch decreased at addition of low concentration of WPI but increased at high concentration. Hot paste stability increased with the addition of WPI in the system, while the relative breakdown decreased when whey proteins were incorporated. It is evident that WPI affected the swelling of starch granules, which ultimately affected the relative breakdown of the starch/WPI mixtures. WPI also strongly affected the thermal behaviour of oat starch, in that peak temperature increased markedly when WPI was added in the system. The addition of WPI exhibited two DSC

endotherms, which are affiliated to the starch gelatinization and β-lactoglobulin denaturation. Enthalpy was increased significantly at 100% WPI/starch mixture in comparison with other combinations. XRD and FTIR analysis assisted in the understanding of long-range and short-range crystallinity order of oat starch/WPI mixtures and supported the thermal analysis results. The XRD study showed that WPI addition increased the relative crystallinity of the mixtures and led to more ordered structure formation in the mixtures. The FTIR analysis supported the DSC and XRD analysis results, as a decrease in IR bands at 1024 cm^{-1} and 1080cm^{-1} suggested an increase in ordered structuration within the WPI/starch mixtures. The scanning electron microscopy also indirectly indicated the increase in gel strength of oat starch gels as WPI were added, a denser honeycomb structure with a dispersed protein layer was observed upon incorporation of WPI.

Overall, whey proteins affected the starch gelatinization properties by reducing the starch swelling, which could be due to less availability of water for the starch or the gelling ability of WPI. The concentration of WPI also played an important role in affecting oat starch properties.

Chapter 7

7 Thermal, pasting and structural studies of oat starch-caseinate interactions

7.1 Introduction

In the previous chapter, the effect of WPI on oat starch was investigated to determine the effects on oat starch, this chapter is focused on casein proteins in the form of CaCN and their effect on oat starch properties.

Starches are generally employed for their thickening and stabilizing ability. Calcium caseinate (CaCN) is normally heat stable, poorly soluble at high temperature and forms reversible viscous gels upon cooling (Srinivasan, Singh, & Munro, 1999). In systems where these two components occur together, the interactions between them need to be understood if synergistic effects are to be utilized, or antagonistic effects prevented, to provide maximum benefits in new or current food products.

Lelièvre and Husbands (1989) examined the effect of sodium caseinate on corn and waxy maize starch and reported that the presence of sodium caseinate increased the viscosity of starch paste. The swelling volume of starch granules was found to be increased in the presence of sodium caseinates with a synergistic effect that lead to shear thinning. The suggested mechanism was the limiting of disintegration of the starch granule through either a reduction in the rate of swelling or by limiting the collapse of the swollen granules due to the action of shear during pasting. Zaleska *et al.* (2001) studied potato starch and casein complexes via electrosynthesis and infrared spectroscopy. The infrared spectra confirmed the interaction of the functional group of starch and casein proteins. The band shift was observed in the region of CO-NH group (~1650 cm^{-1}), NH moiety (~1530 cm^{-1}) and OH group bending modes of starch and casein between 1370-1270 (cm^{-1}), which indicated possible interactions between the

amino groups of the milk protein and the phosphate groups of the potato starch. Noisuwan *et al.* (2007) explored the effect of sodium caseinate on normal and waxy rice starch granules. The authors reported that the swelling onset temperature was increased from 56.2 ^{o}C to 58.6 ^{o}C by the addition of sodium caseinate.

Extensive studies have been carried out on milk proteins interactions with different starches but, no scientific research has been reported on interactions of calcium caseinate with oat starch. It is important to understand the mechanisms, interactions and synergistic effects that can provide maximum benefit to a food product. Hence, the resultant studies will help explain the characteristics and behaviour of these interactions in various environments and this will in turn be useful for the emerging dairy-cereal product manufacturing industry.

7.2 Material and Methods

7.2.1 Materials

This section is described in 3.1.

7.2.2 Sample preparation

The mixtures with oat starch and calcium caseinate (CaCn) were prepared as 1:0 (0% CaCN), 1:0.25 (25% CaCN), 1:0.5 (50% CaCN), 1:0.75 (75% CaCN) and 1:1 (100% CaCN) and starch concentration was kept constant 10 % (w/w) for all the experiments.

7.2.3 Rheometer Pasting properties

This section is described in 3.2.13.

7.2.4 DSC analysis

This section is described in 3.2.14

7.2.5 X-ray diffraction (XRD) analysis

This section is described in 3.2.15

7.2.6 Fourier transform infrared (FTIR) spectroscopy

This section is described in 3.2.16

7.2.7 Gel Scanning electron microscopy (SEM)

This section is described in 3.2.17

7.2.8 Statistical analysis

This section is described in 3.2.19

7.3 Results and Discussion

7.3.1 Pasting properties

The pasting behaviour of oat starch containing different calcium caseinate concentrations is shown in Table 7-1. The addition of CaCN increased the RVA parameters significantly ($p < 0.05$) in comparison with the pure oat starch sample. Peak and trough viscosities increased on the addition of 50%, 75% and 100% CaCN concentration to oat starch, however, these values were not significantly affected by caseinate when low concentrations (25%) of CaCN was added. The higher the CaCN concentrations, the higher were the breakdown, final and setback viscosities, especially above the level of 50% CaCN addition. The peak viscosity is attributed to starch swelling and water absorption during gelatinization process and shows an equilibrium between swelling and consequent disruption of starch granules during shearing, while the increase in peak viscosity due to the addition of CaCN is attributed to an overall increase in swelling volume of starch granules.

Similar results were obtained by Lelièvre and Husbands, (1989) when studying sodium caseinate/corn starch mixtures. A study of Bertolini et al. (2005) also reported that the addition of sodium caseinate with different starches (Cassava, potato, corn, wheat and rice) increased the peak viscosity and final viscosity in all the mixtures except for the potato starch. Noisuwan et al. (2007) also reported the similar pattern of peak viscosity and final viscosity in the sodium caseinate and rice starch mixtures.

Breakdown viscosity simply suggest the extent of starch molecules disruption and usually a difference of peak and trough viscosities, to better understand the pattern, stability ratio and relative breakdown were derived from the RVA parameters. The stability ratio was the ratio of trough viscosity to peak viscosity and relative breakdown was the ratio of breakdown viscosity to setback viscosity. The paste stability is used to indicate the ability of a gel/paste to withstand the shear and high temperature during pasting. The stability ratio was decreased significantly ($p < 0.05$) at high concentration of caseinates, markedly above the 50% CaCN, while increased at 25% CaCN/starch mixture.

Table 7.1 Pasting parameters of oat starch with CaCN

Samples	Peak Viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final (cP)	Setback (cP)	Stability Ratio	SetBack Ratio	Relative Breakdown
Oat Starch	3218.4±20.8^{d}	1957.82±12.65^{c}	1260.57±8.17^{d}	4948.3±18.6^{c}	2990.47±6.07^{b}	0.608±0.000^{b}	2.52±0.006^{a}	0.421±0.001^{e}
CaCN 25%	3145.8±28.0^{d}	2007.34±13.51^{c}	1138.4±14.5^{e}	4157.9±16.9^{e}	2150.57±3.40^{e}	0.638±0.001^{a}	2.07±0.005^{e}	0.529±0.005^{d}
CaCN 50%	3850.0±22.1^{c}	2281.53±12.45^{b}	1568.50±9.63^{c}	4838.00±18.6^{d}	2556.48±6.10^{d}	0.592±0.000^{c}	2.12±0.003^{d}	0.613±0.002^{c}
CaCN 75%	4169.1±22.5^{b}	2307.89±13.11^{b}	1861.24±9.44^{b}	5104.60±20.1^{b}	2796.67±6.95^{c}	0.553±0.000^{d}	2.21±0.003^{b}	0.665±0.001^{b}
CaCN 100%	5484.6±23.1^{a}	2682.06±11.45^{a}	2802.50±11.64^{a}	5814.4±17.9^{a}	3132.38±6.41^{a}	0.489±0.000^{e}	2.16±0.002^{c}	0.894±0.001^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

High breakdown viscosity and low stability ratio suggested the low shear resistance of starch molecules at the caseinate concentration more than 50%, while at low calcium caseinate concentration (25% CaCN), breakdown viscosity decreased and showed a high stability ratio in comparison with oat starch alone. This result suggested that at 25% CaCN addition, starch granules showed a reduced swelling and disruption during pasting, which is also described by Shafie et al. (2016). The high concentration of caseinates did not affect the swelling and disruption of starch molecules and suggested that the protein network did not contributed in resistance to mechanical shearing of oat starch/CaCN mixtures, which was probably due to self-association of caseinates at high concentrations and not affecting the starch molecules. The stability ratio might be useful in determining the quality of hot served products such as soups and porridge, where the desired hot paste viscosity is a crucial factor (Joshi et al., 2014). The Setback viscosity and setback ratio indicated retrogradation behaviour of oat starch/CaCN mixtures after gelatinization, which is a re-association of leached starch molecules such as amylose and amylopectin. The setback viscosity decreased significantly ($p < 0.05$) at 25%, 50% and 75% concentration of CaCN, while an increased at 100% CaCN in comparison with oat starch. This indicated the reduced restructuring of amylose molecules leading to less retrogradation, which is a desirable character for some food products, such as yoghurt and bread. Setback ratio, which is a ratio of setback to trough viscosity, showed a decrease in CaCN/starch mixtures in comparison with starch aloneIn summary, the effect of calcium caseinate addition on oat starch pasting properties are highly dependent on concentration of caseinates in the mixture. At low level of caseinate (25% and 50%), it was evident that CaCN affected the starch swelling and disruption more in comparison to high CaCN concentration. CaCN supported the high viscosity of the mixtures which was due to swelling volume of starch fraction and seemed to have no effect on starch

Table 7.2 Thermal analysis parameters of oat starch and oat starch/CaCN mixtures

Samples	T_o* (◦C)	T_p* (◦C)	T_c* (◦C)	ΔT (◦C)	ΔH (j/g)
Oat Starch	56.35±0.070^{c}	61.200±0.283^{d}	67.000±0.283^{b}	10.650±0.354^{a}	12.040±0.085^{a}
CaCN 25%	57.385±0.091^{b}	62.130±0.028^{c}	68.360±0.014^{a}	10.975±0.106^{a}	9.450±0.212^{b}
CaCN 50%	57.800±0.0141^{b}	62.835±0.021^{b}	68.715±0.021^{a}	10.915±0.120^{a}	8.383±0.307^{c}
CaCN 75%	58.665±0.0163^{a}	63.345±0.007^{ab}	67.130±0.028^{b}	8.465±0.191^{c}	5.860±0.024^{d}
CaCN 100%	58.930±0.113^{a}	63.665±0.021^{a}	68.390±0.056^{a}	9.460±0.056^{b}	5.428±0.280^{d}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

7.3.2 Thermal Properties

The DSC parameters (T_o= onset temperature of gelatinization endotherm; T_p = peak temperature; T_c = conclusion; $\Delta T = T_c - T_o$ and ΔH= enthalpy change) of oat starch alone and oat starch/CaCN mixtures are shown in Table 7-2 and Figure 7-1. In the present study, a single endothermic peak was observed. The addition of CaCN to oat starch showed a shorter thermogram than oat starch/water mixture alone. The width and height decreased with increasing CaCN concentration (Figure 7-1). The DSC thermogram of CaCN/starch mixtures was appeared to shift slightly to the right and the shift was proportional to the amount of added CaCN concentration up to 50%. T_o (onset temperature) increased by 1.45 ◦C and T_p (peak temperature) by 1.63 ◦C on the addition of 50% CaCN in comparison with control. Table 7-2, indicates that the addition of CaCN increased the T_o and T_p significantly ($p < 0.05$) in comparison with control, however, there no significant difference was observed between T_o of CaCN 25% and 50%, similarly between 75% and 100% CaCN. There was no significant difference observed in peak temperature (T_P) of 75% CaCN and 100% CaCN/starch mixtures. As discussed earlier in the chapter, the addition of high CaCN concentration (75% and 100%), decreased the gelatinization range (ΔT) significantly and this is supported by the decrease in width and height of peak (Figure 7-1). There was also a decrease in enthalpy (ΔH) due to the addition of CaCN, but there was no significant difference in ΔH observed between CaCN 75% and CaCN 100%. The gelatinization temperature of oat starch increased with increasing concentration of CaCN, this was more prominent at high concentrations of CaCN. However, the enthalpy decreased as CaCN concentration was increased. The results obtained are in agreement with the results obtained by Erdogdu, Czuchajowska, and Pomeranz (1995), who studied the thermal behaviour of casein /wheat starch mixtures. They observed the increase in onset, peak and end temperature, similarly a decrease in enthalpy.

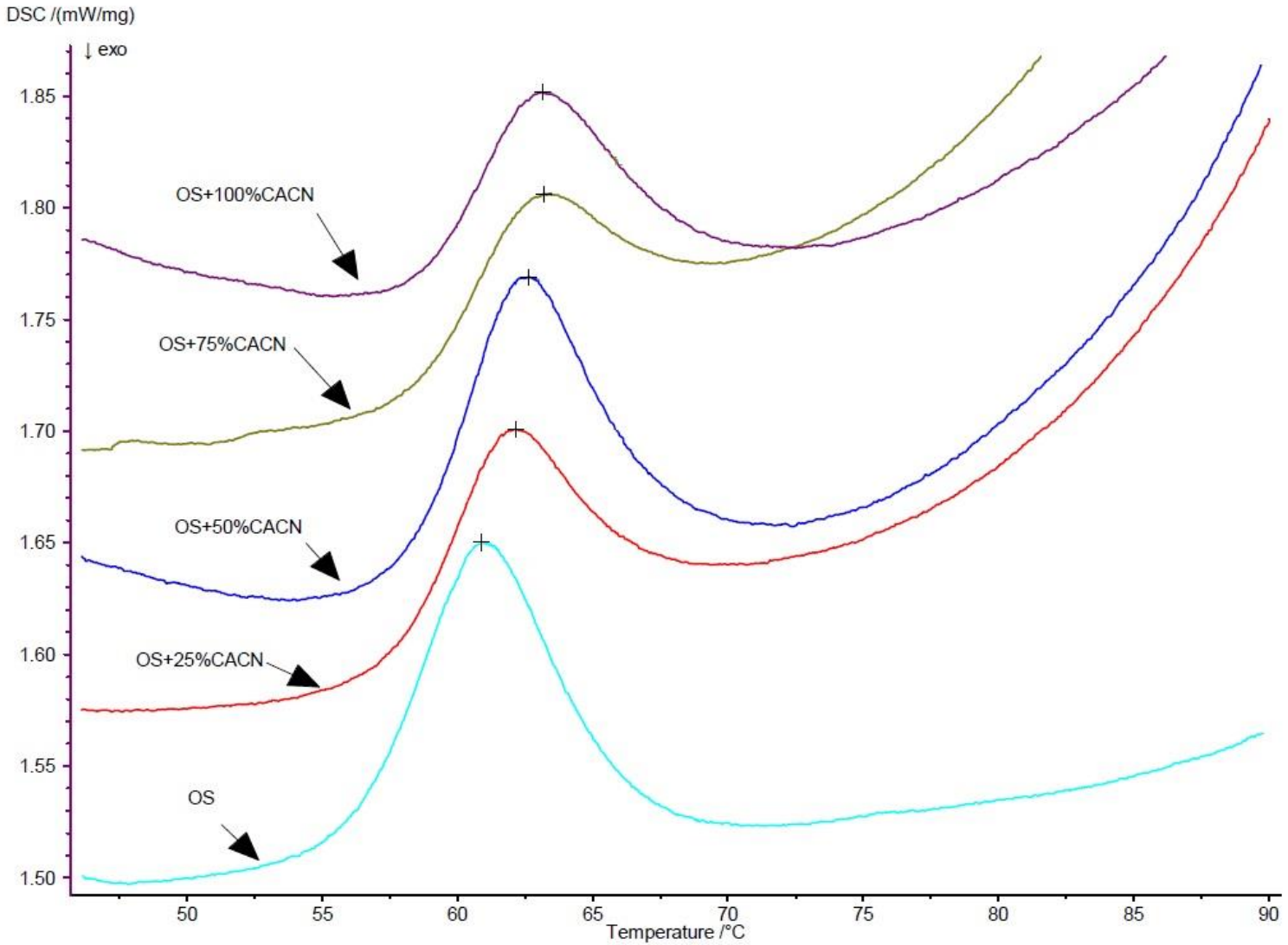


Figure 7-1 DSC thermograms of oat starch and oat starch/CaCN mixtures

Bertolini *et al.* (2005) also suggested that sodium caseinates increased onset temperature, gelatinisation temperature and end temperature of the gelatinisation process in all the starches. This is explained by the lack of enough water to hydrate the starch, which can hinder the gelatinization process. Noisuwan et al. (2008) observed a similar effect of sodium caseinates on rice starch. Peak and onset temperature were increased upon addition of sodium caseinates. Another mechanism which can affect the starch gelatinization is adsorption of the caseins onto the starch granule surfaces. Caseins can form large aggregates of proteins around starch granule surface specially β-caseinates, which is due to their micelle forming capacity (de Bont et al. 2002; Olsson et al. 2000; Olsson et al. 2002; Ravindra et al. 2004; Vu Dang et al. 2009). The hypotheses of penetration and physical adsorption of proteins on starch granules was postulated by Noisuwan et al., (2011); Kett et al., (2013). However, these studies showed only the penetration of α-caseinate and the adsorption of casein proteins and their subfractions on starch granule surface. This could be due to bonding between endogenous lipids and proteins of starch present on its surface and exogenous dairy protein. Consequently, the adsorbed proteins might restrict water penetration into the starch granules delaying the gelatinisation.

7.3.3 FTIR analysis

FTIR spectroscopy was used to examine oat starch/CaCN gels, the vibrational frequencies between atomic bonds in mixtures are recorded as absorption peaks in Figure 7-2 and 7-3. Hydrated starch gels mixtures showed pronounced peaks at 3000-3700 cm^{-1}, 2000-2250 cm^{-1}, 1500-1750 cm^{-1} and 1000-1200 cm^{-1}, while starch/CaCN mixture showed some additional bands at amide I and amide II region, peaked at 1638 cm^{-1} and 1547 cm^{-1}. The major bands contributed by starch can be observed in the region 1000-1200 cm^{-1}, arising from C- O, C-C

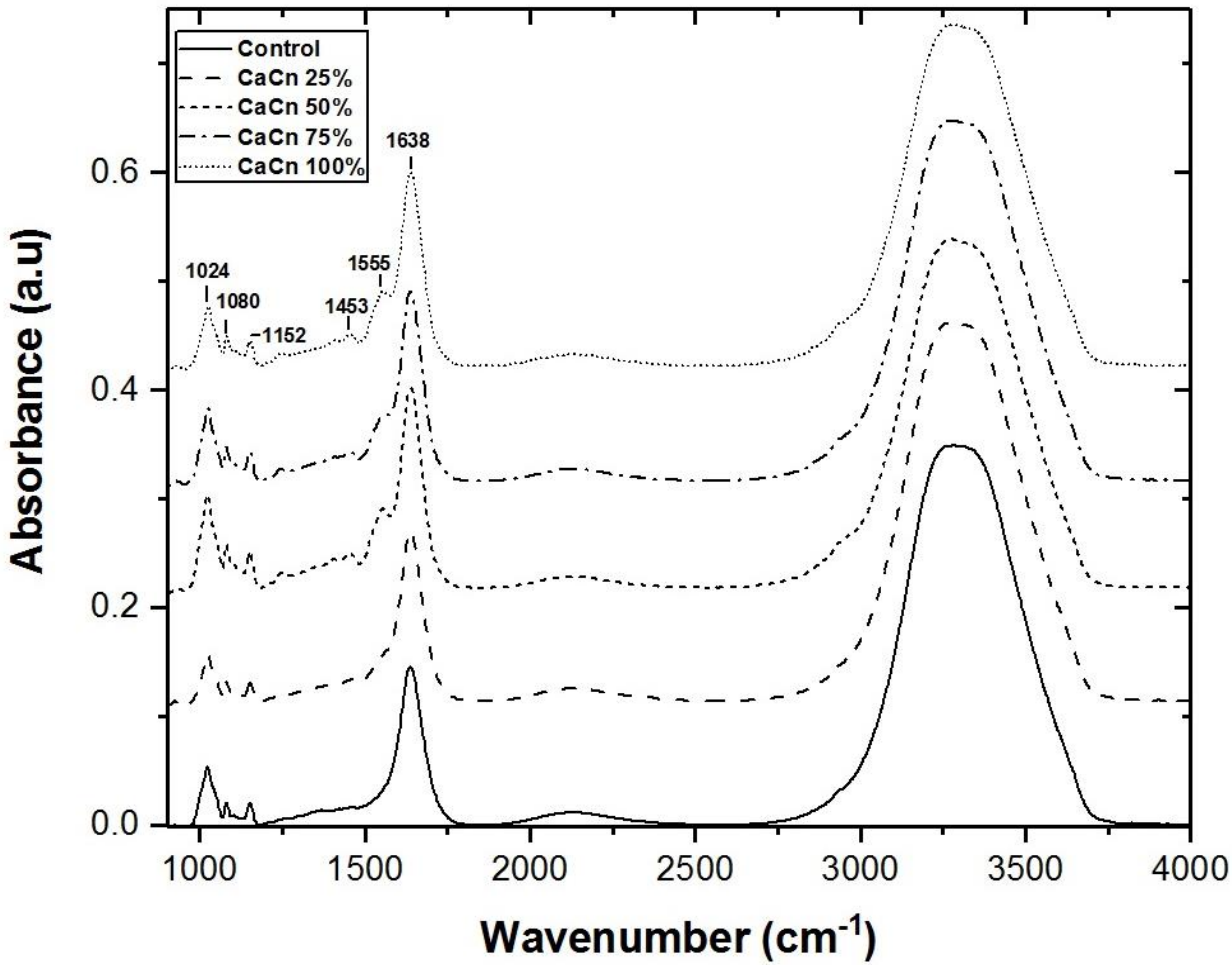


Figure 7-2 FTIR spectra of oat starch with CaCN

and C-O-H stretching and bending (Warren et al., 2016). The changes in the region 1000-1200 cm^{-1} are assigned to change in starch structure, specifically peaks at 1024 cm^{-1} is an indicator of gelatinized starch, where semi-crystalline structure of starch was fully disintegrated into amorphous region and attributed to C-O-H strain vibrations (van Soest et al., 1995). As can be observed from Figure 7-3, the IR bands intensity at ~1024 cm^{-1} and ~1080 cm^{-1} increased by the addition of CaCN in the system except 25% CaCN inclusion, where it decreased. The IR bands at ~1022-1024 cm^{-1} have been suggested to be associated with amorphous structure of starch (Zeng et al., 2015). The FTIR spectra indicated that the addition of CaCN increased the absorbance intensity at 1024 cm^{-1}, which suggested the increased amorphous order of starch or decreased crytallinity. The increase in the intensity of the band at 1024 cm^{-1} shows a decrease in crystallinity (Rivera-Corona et al., 2014; van Soest et al., 1995). This increase was maximum at CaCn 50%, followed by 75% and 100% CaCN addition. However, CaCN 25% showed a decrease in 1024 cm^{-1}, which suggested a more ordered structure of starch. The absorbance at 1080 cm^{-1} is also assigned to stretching of C-O,C-C and O-H bonds and an alternative indicator of ordered structure, the band at 1152 cm^{-1} also suggested the amorphous order of starch (Rubens et al., 1999; Warren et al., 2016), which showed an increase in intensity leading to conclude that CaCN addition in oat starch resulted into more amorphous state of starch and indicates that CaCN did not affect the gelatinization process much.

The amide I band region centred at 1638 cm^{-1}, showed an increment with the addition of CaCN, and attributed to stretching vibrations of the CO-NH group. The signal at 1547 cm^{-1} corresponded to the amide II region contributing to peptide bond group vibrations (N-H), it was suspected that intra and inter-molecular hydrogen

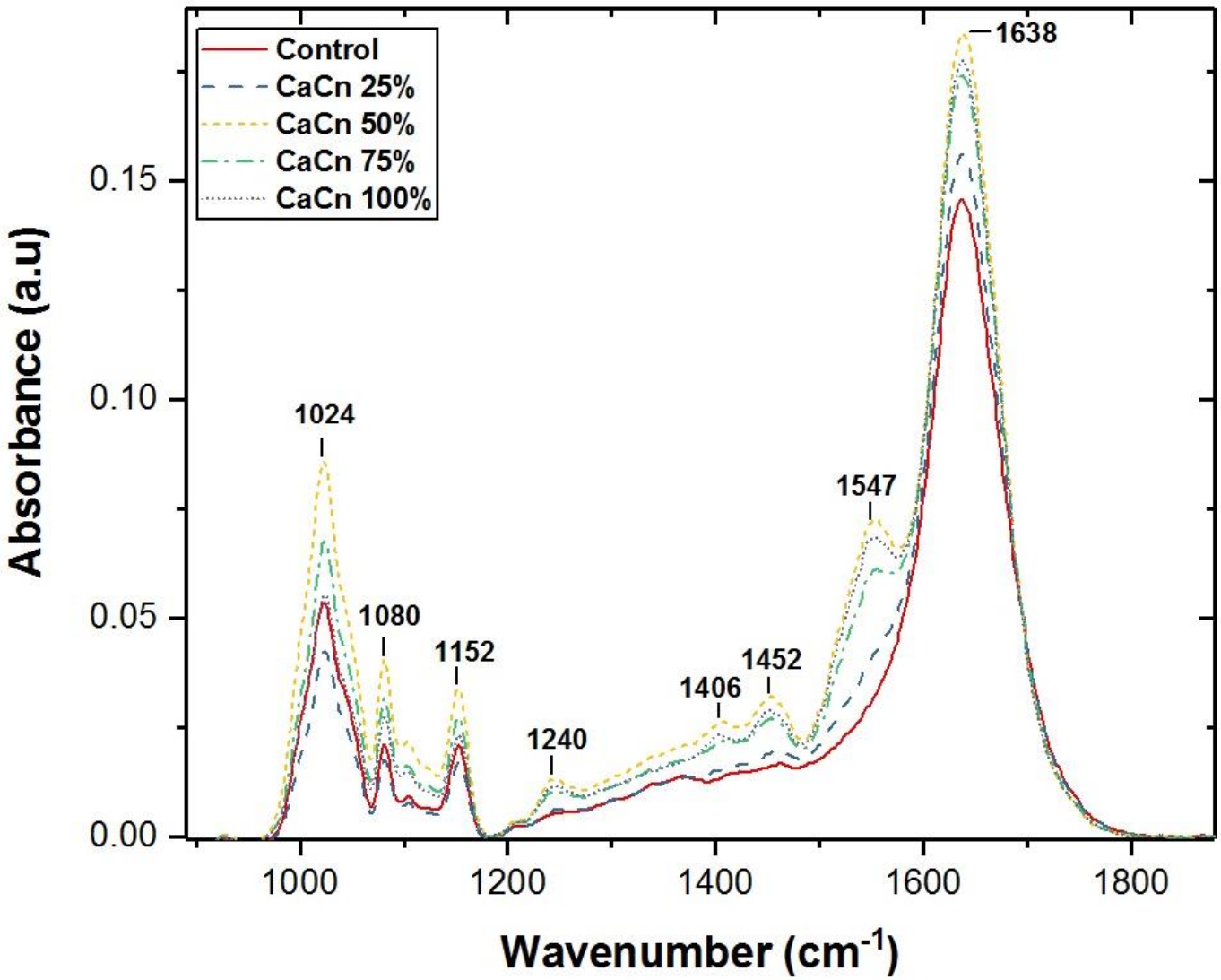


Figure 7-3 FTIR spectra showing amide I (1600-1700) and amide II (1400-1600) region with starch sensitive bands (1000-1200)

bonding and hydrophobic interactions can affect the band intensity, which might be due to casein/oat starch interactions. Similar results were reported by Sun et al. (2016), where bands at ~1530 cm^{-1} were shifted due to hydrogen bonding. The vibration absorbance bands at 1406 cm^{-1} and 1452 cm^{-1} corresponds to -CH_2 moieties of amino acid side chains (Barth, 2000). The vibrational intensity at ~1240 cm^{-1} was also observed and attributed to bending modes of OH groups of starch and casein. Similar bands were reported by Zaleska, Ring, and Tomasik (2001) in a study of acid casein and potato starch. Finally, it can be concluded from the FTIR studies that CaCN addition affected the short range ordered structure of oat starch and the internal structure was became more amorphous specially at 50%,75% and 100% CaCN concentration, which suggest that CaCN had not much effect on starch gelatinization. This might be due to self-gelling/aggregation capacity at low temperature (50-60 ◦C) as CaCN is less heat stable, CaCN also had a low water binding and hydration capacity which can leave enough water available for starch to gelatinize (Singh, 2011).

7.3.4 XRD analysis

The XRD pattern of gelatinized oat starch and CaCN/starch mixtures are presented in Figure 7-4. The two broad peaks at 20^o and 35^o represented the amorphous nature of starch, which is an indicator of successful gelatinization of oat starch and complete disruption of ordered semi-crystalline structure under shear and high temperature. González-Gutiérrez et al. (2011) also reported the loss of crystalline peaks in gelatinized samples of albumen/potato starch mixtures. The addition of CaCN did not affect the diffraction pattern and crystallinity of oat starch, however showed a slight reduction in the ordered structure in comparison to oat starch alone. Hence, the addition of CaCN resulted in a more amorphous nature of CaCN/starch mixtures. The relative crystallinity (RC%) of CaCN/starch remained almost similar to control, which suggested no effect on starch gelatinization. There was no effect of increasing CaCN

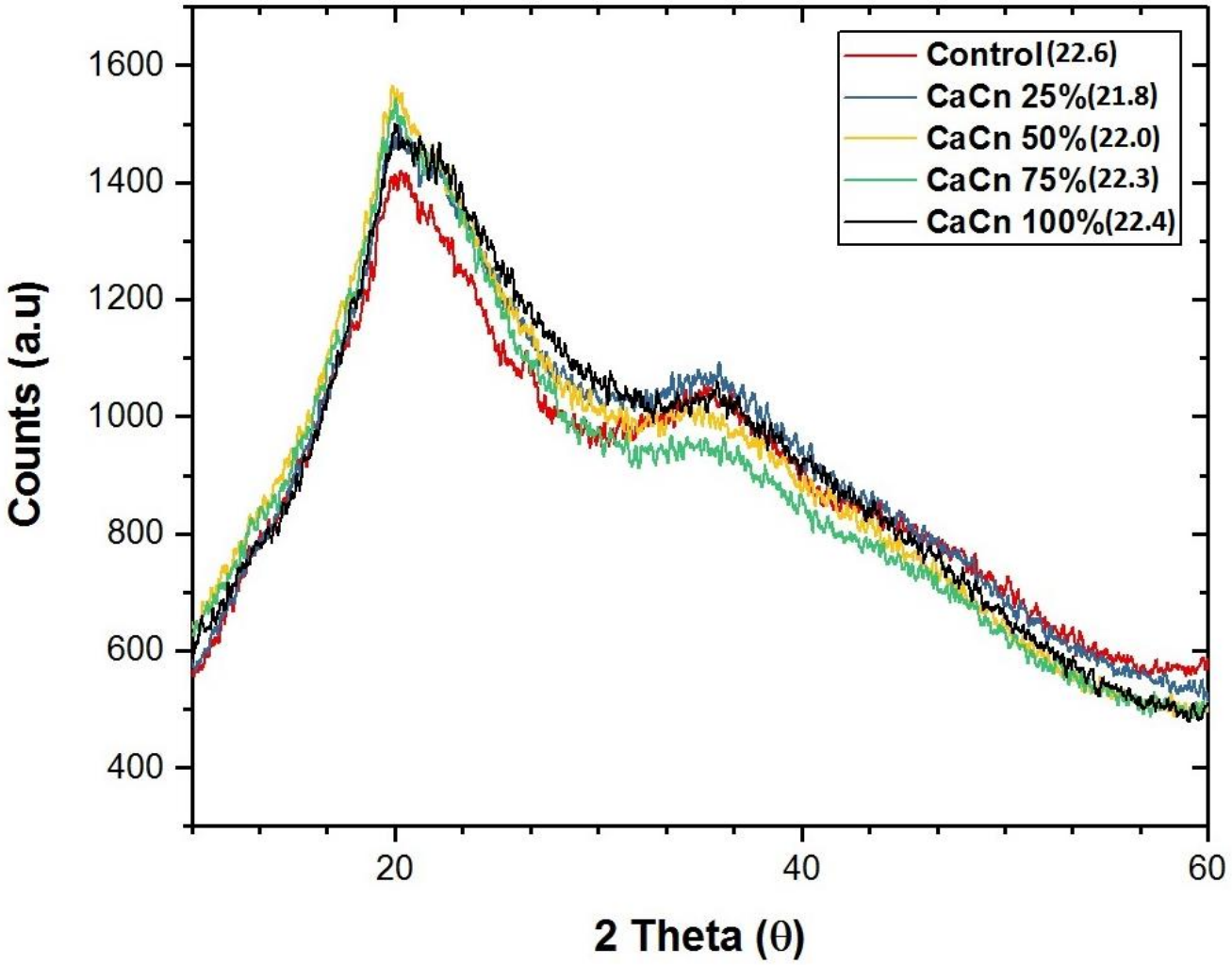


Figure 7-4 XRD diffractogram of oat starch/CaCN freeze-dried gels

concentration on RC (%) observed in the samples. Rivera-Corona et al. (2014) studied the effect of cactus mucilage addition on sorghum starch and observed a reduction in crystallinity of sorghum starch. They suggested that this might be due to physical interactions between starch (OH group) and cactus mucilage (NH, OH) groups. Similarly, XRD diffraction pattern of CaCN/oat starch suggested that by heating, the CaCN/starch mixture resulted in a slight increment of an amorphous disorderly state. The results of XRD was in agreement with the increased bands at 1024 cm^{-1} and 1080 cm^{-1} studied by FTIR as discussed earlier in section 7.3.3.

7.3.5 Scanning electron microscopy

The SEM micrographs of freeze-dried oat starch-CaCN gels at different protein concentrations are presented in Figure 7-5. The micrographs show a typical starch/protein composite gel internal structure, and similar to micrographs reported by Li, Yeh, and Fan, (2007) and Joshi et al., (2014) for soy protein/corn starch and lentil protein/lentil starch gels respectively. Similarly, the SEM micrographs of milk proteins and polysaccharides were reported by Azim et al. (2010); Colombo, Ribotta, and León (2014) and Q. Sun and Xiong (2014). Both native oat starch and CaCN/starch blends formed three-dimensional honeycomb network which suggests effective starch gel formation. The oat starch gel structure seemed to have large pore size and a shallow pore structure, which indicated strong starch gel formation (Figure 7-5, a-b). The microstructure of oat starch/CaCN gels are presented in Figure 7-5(c-j), indicating 25% CaCN/starch (c-d), 50% CaCN/starch (e-f), 75% CaCN/starch (g-h) and 100% CaCN/starch (i-j) mixtures. As it can be observed from the Figure 7-5(c-j), upon incorporation of CaCN, the structure of WPI/starch gels became denser and reduction in the pore size of the structure was observed. A denser and shallow pore depth structure was exhibited in Figure 7-5 (g-j), which indicated that CaCN molecules can form protein gel and modulate the oat starch gel

structure by dispersion of a protein layer over starch network. The micrographs showed a more denser pores in the gel network in comparison with WPI, that might be due to less heat stability of calcium caseinates and gelation tendency at low temperature (50-60 ^{o}C) (Singh, 2011).

7.4 Correlation between WPI and CaCN affecting oat starch properties

In chapter 6, WPI showed significant effect on the starch granules by restricting the swelling due to unavailability of water to starch molecules. While, CaCN showed less prominent effect on starch, this might be due to its lower heat stability, gelling ability. The formation of large casein aggregates, which might lead to hydrophobic regions, resulting into more water available for starch swelling. WPI have more water solubility and gelling ability, which is due to thermal denaturation of WPI amino acid chains. The denatured chains lead to disulphide linkages between the chains and more gelling.

7.5 Conclusion

In Summary, CaCN affected the oat starch pasting, thermal and structural properties. The effect of calcium caseinate addition on oat starch pasting properties are highly dependent on concentration of caseinates in the mixture. At low levels of caseinates (25%), peak viscosity was not significantly different from the oat starch alone, while as the CaCN concentrations were increased, the higher were the breakdown, final and setback viscosities, especially above the 50% CaCN addition. Setback and stability ratio were decreased when CaCN added at high concentration. An increase in peak temperature (T_P) was observed in DSC analysis by addition of CaCN in oat starch, furthermore no huge effect on peak temperature was observed among high CaCN concentrations. Enthalpy enthalpy was decreased as CaCN concentration was increased leading to shorter peak thermographs. XRD study showed that CaCN addition have

no or little effect on the relative crystallinity of the mixtures and led to more amorphous structure formation of mixtures. FTIR analysis supported the XRD analysis results, as a increase in IR bands at 1024 cm^{-1} and 1080cm^{-1} suggested the increase in amorphous structuration of the CaCN/starch mixtures.

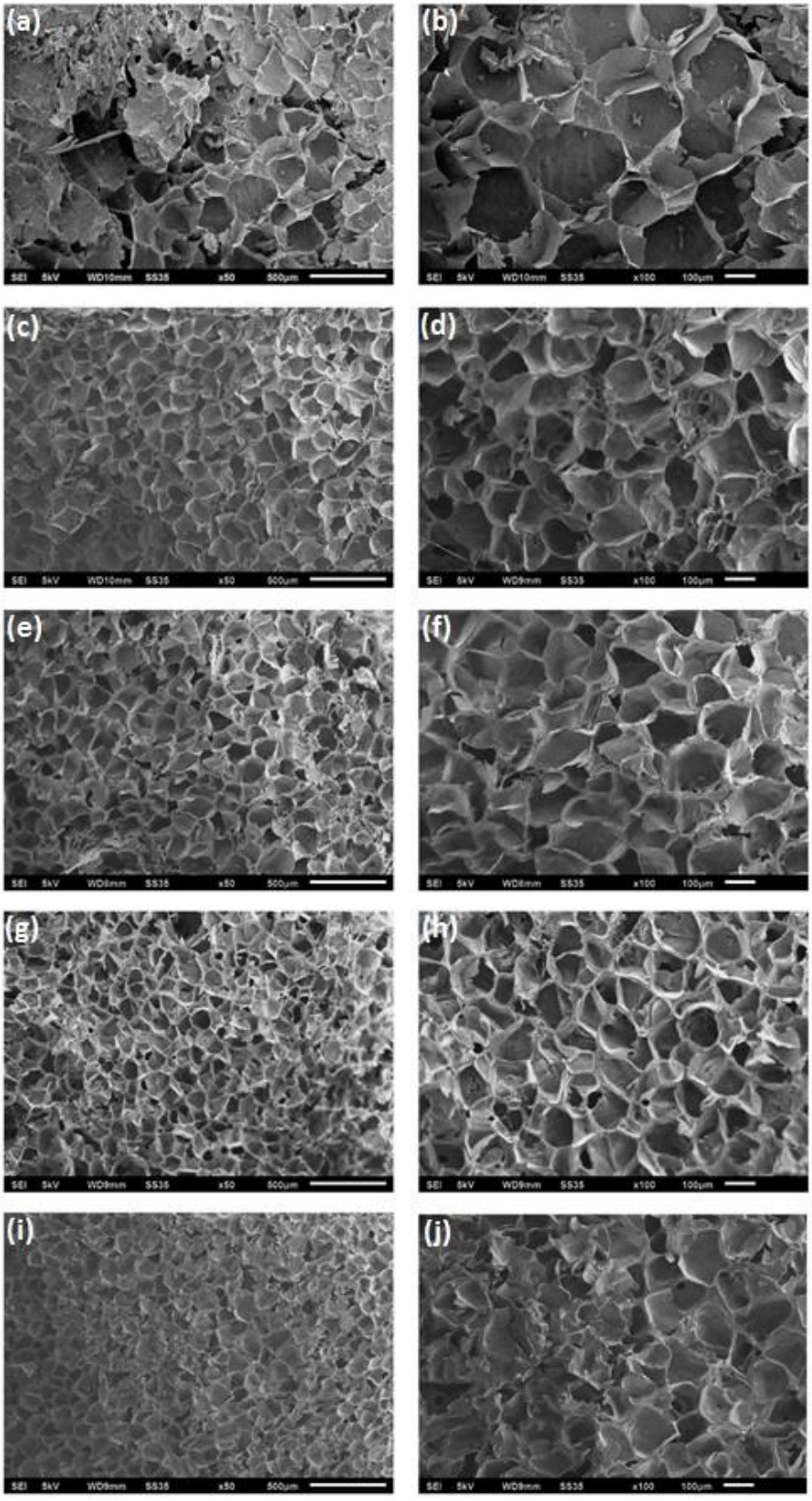


Figure 7-5 SEM micrographs of oat starch/CaCN freeze dried gels indicating oat starch (a-b), 25% CaCN/starch (c-d), 50% CaCN/starch (e-f), 75% CaCN/starch (g-h) and 100% CaCN/starch (i-j)

Chapter 8

8 Co-gels of whey protein isolate with oat Starch: A study of rheological and textural properties

8.1 Introduction

In chapter 6, the effect of WPI with oat starch in terms of thermal, pasting and structural characteristics were studied. This chapter was aimed to study the rheological behaviour of addition of WPI on oat starch gels, as WPI was found to affect starch gelatinization, it is evident that it will affect the gelation behaviour of oat starch.

Starch and milk proteins are used to improve textural properties in various dairy-based products such as custards, yoghurts and processed cheeses (Brown, McManus, & McMahon, 2012; Oh, Anema, Wong, Pinder, & Hemar, 2007). The fact that common starch sources (wheat, corn, rice, potato and tapioca) and milk proteins are extensively investigated, however, studies on oat starch and their interactions are relatively scarce. Whey protein isolate (WPI) have good gelling properties and could affect starch gelatinisation, greater than caseins. A gelatinized starch gel is a suspension of swollen granules, starch remnants and three- dimensional network formed by leached amylose and amylopectin. A oat starch gel behaves like viscoelastic solid, pseudoplastic and thixotropic in nature (Berski et al. 2014; Doublier et al. 1987).

Noisuwan et al. (2009) reported a slight increase in storage modulus (*G'*) with the increase of oscillatory frequencies in both normal and waxy rice starches, but they experienced an overall weak gel structure in small deformation rheological measurements.

The interaction of starch and protein can affect the macroscopic properties of food such as: flow, stability, texture and mouth feel.

Although extensive literature is available on the rheology of mixed WPI and various starch dispersions, not much information on WPI and oat starch dispersions were studied, especially whether they exhibit synergistic or antagonistic rheological behaviour. Therefore, an attempt was made to elucidate the rheological and textural properties of WPI and oat starch mixtures by using steady, dynamic, time dependent, three interval thixotropic and hysteresis loop tests.

8.2 Material and Methods

8.2.1 Materials

This section is described in 3.1.

8.2.2 Sample preparation

The mixtures with oat starch and whey protein isolate (WPI) were prepared as 1:0, 1:0.25, 1:0.5, 1:0.75 and 1:1 and starch concentration was kept constant 10 % (w/w) for all the experiments. Gel samples were prepared as described in pasting section and then various rheological test were performed at 25 °C.

8.2.3 Rheological properties

8.2.3.1 Steady state and dynamic rheology

This section is described in 3.2.18.1

8.2.3.2 Thixotropy analysis

This section is described in 3.2.18.2

8.2.3.2.1 Hysteresis test

This section is described in 3.2.18.2.1

8.2.3.2.2 Three interval thixotropy test (3ITT)

This section is described in 3.2.18.2.2

8.2.3.2.3 Time dependent

This section is described in 3.2.18.2.3

8.2.4 Textural properties

This section is described in 3.2.12.1

8.2.5 Statistical analysis

This section is described in 3.2.19

8.3 Results and Discussion

8.3.1 Steady state and dynamic rheology

The continuous shear stress flow curves of the oat starch and oat starch/WPI mixtures are presented in Figure 8-2. The flow curves of oat starch and mixtures were fitted by the Power law and Herschel-Bulkley models, and the values are presented in Table 8-1. The increase in shear stress (σ) on increasing shear rate (s^{-1}) indicated a strong shear thinning or pseudoplastic behaviour of oat starch gels, which is also reported by Sikora, Kowalski, and Tomasik (2008). The flow behaviour index of $n<1$ also supported the shear thinning behaviour of oat starch and mixtures (Bashir, Jan, & Aggarwal, 2017; Lu et al., 2012).

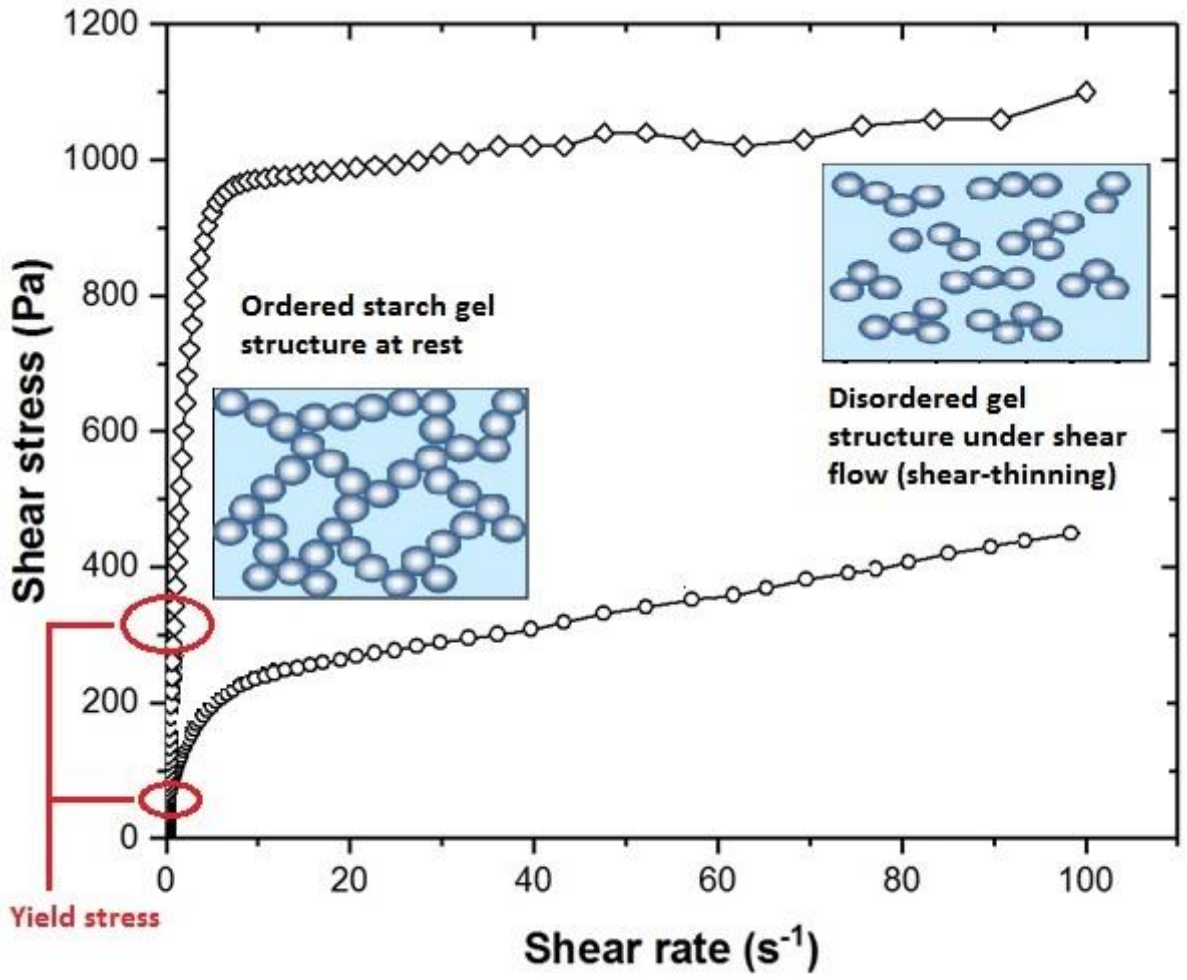


Figure 8-1 Schematic representation of starch gel behaviour under shear flow

Both power law and H-B model showed similar flow parameters of oat starch and starch/WPI mixtures. Flow behaviour index (n) calculated by both models, was significantly increased ($p < 0.05$) by the addition of 25% WPI and decreased by higher concentration of WPI (50%, 75% and 100%) in comparison with oat starch . The decrease in flow index at high concentration of WPI (above 25%) showed increased shear thinning behaviour of mixtures in comparison with oat starch alone and might be due to increased protein continuous phase, therefore limiting the leached amylose three-dimensional restructuring of network upon cooling. The schematic possible structure behaviour of starch gels is shown in Figure 8-1. Consistency index (K) represented the magnitude of viscosity in terms of consistency (Sikora et al., 2007), the consistency index (K) derived from both models, increased significantly when high

concentration (50%, 75% and 100%) of WPI was added, while decreased at 25%.

Table 8.1 Steady state parameters of oat starch/WPI mixtures

Samples	Power Law		H-B Model		
	n	K (Pa s^n)	n	K (Pa s^n)	σ_0 (Pa)
Oat Starch	0.214±0.004^{b}	189.67±0.77^{d}	0.181±0.009^{b}	233.69±8.67^{c}	50.86±0.85^{d}
WPI 25%	0.260±0.003^{a}	119.74±1.62^{e}	0.262±0.011^{a}	115.91±6.42^{d}	51.66±1.35^{d}
WPI 50%	0.139±0.002^{d}	215.67±0.84^{c}	0.155±0.004^{bc}	236.49±2.88^{c}	66.89±0.81^{c}
WPI 75%	0.158±0.003^{c}	232.37±3.18^{b}	0.139±0.004^{c}	338.90±5.46^{b}	123.18±1.51^{b}
WPI 100%	0.151±0.004^{cd}	586.48±2.82^{a}	0.149±0.003^{c}	805.97±6.17^{a}	316.98±1.82^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

This indicated that at high concentration of WPI, starch gels were more viscous than low concentration and represented a weak gel behaviour when sheared. At 25% WPI concentration, oat starch/WPI gels acted like a less shear thinning and liquid like material. Vu Dang et al. (2009) also reported that the viscoelastic behaviour of WPI/cross linked waxy maize starch is transformed from a solid like to a liquid like form when the WPI concentration is increased, this suggested increased shear thinning behaviour at high WPI concentration. The yield stress (σ_0) also increased significantly upon the increase of gradual concentration of WPI except at 25% WPI. The increase in yield stress and decrease in flow index (n) indicated a complex type of gel which is highly dependent on exerted force, this type of starch/WPI gel will show more strength at low deformation energies, while weak gel behaviour at high deformation force.

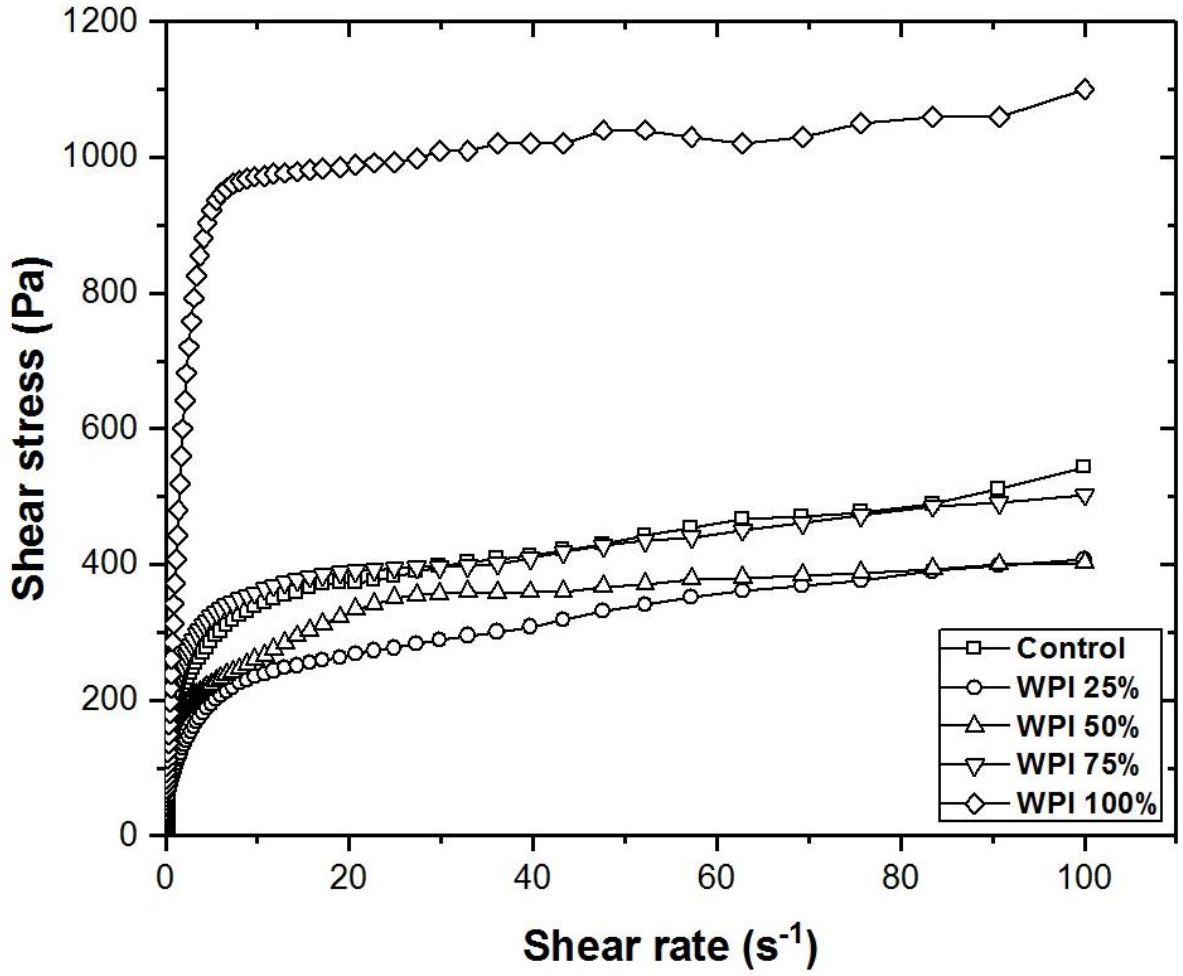


Figure 8-2 Shear stress values as a function of shear rate in oat starch/WPI mixtures

The amplitude sweep measurements for gels of oat starch and starch/WPI mixtures are reported in Figure 8-3 to 8-5. The amplitude sweep parameters at 1% strain and at constant frequency of 1 Hz are illustrated in Table 8.2. Elastic modulus (G') and viscous modulus (G") represents the elastic and viscous components of viscoelastic material. As can be illustrated from Table 8.2, at low strain amplitude sweep (1%), G' was higher than the G", which is an indication of elastic nature of starch gels. However, G' decreased significantly by the addition of WPI in comparison with oat starch except for 100% WPI. This suggests weakening of starch

gels by the addition of WPI, which might be due to WPI effect on starch gelatinisation. Complex modulus (G*) also showed the

Table 8.2 Amplitude sweep parameters of oat starch/WPI mixtures at 1% strain and at constant frequency of 1 Hz

Samples	σ (Pa)	G' (Pa)	G" (Pa)	tan δ	G* (Pa)
Oat Starch	6.10±0.141^{b}	604±8.49^{b}	102.50±2.12^{bc}	0.170±0.001^{e}	97.35±1.48^{b}
WPI 25%	4.65±0.035^{c}	450.50±3.54^{c}	105.50±3.54^{bc}	0.237±0.001^{d}	72.10±2.83^{c}
WPI 50%	3.85±0.01^{d}	371.50±2.12^{d}	98.50±2.12^{c}	0.268±0.001^{b}	60.55±1.34^{d}
WPI 75%	3.95±0.02^{d}	378±4.24^{d}	110±1.41^{b}	0.290±0.000^{a}	61.65±2.19^{d}
WPI 100%	14.95±0.35^{a}	1456.50±4.95^{a}	363.50±2.12^{a}	0.249±0.007^{c}	238±2.83^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

overall resistance to deformation and indicates rigidity or stiffness, which decreased significantly by the addition of WPI (25%, 50% and 75%) in comparison with oat starch alone and increased for 100% WPI/starch mixture. No significant difference in G* and G' was observed at the concentration of 50% and 75% WPI mixtures. The results indicated that the addition of WPI up to 75%, affected the starch swelling and gelatinisation, which in turn ultimately decreased the gel elasticity/strength. However, at 100% WPI, the increase in G' and G* is due to gelation of the WPI at high concentration. Similar results were reported by Noisuwan et al. (2009), where rice starch/WPI mixtures were investigated. tan δ is a ratio of

viscous modulus to elastic modulus, the values increased upon the addition of WPI, indicated elastic-solid like strong gel to viscous-liquid like gel transition or weak gel behaviour (Figure 5). From Figure 3 and 4 it can be clearly seen that the addition of WPI affected the values of G' and G" and at low strains gels were elastic (G'>G"), but at high strains values G" became higher than G', indicating that elastic gels behaved as viscous materials or liquids. To understand the gel behaviour at large deformation, the maximum strain (γ_{max}) was calculated, which is defined as the strain at which G'=G", at this point maximum stress (σ_{max}) was also recorded. The point where G' and G" becomes equal called as "Flow point". Flow point is basically a representation of transition phase, where elastic/gel like material starts behaving like viscous material. The results of flow point parameters are reported in Table 8.3.

Table 8.3 Flow point parameters of oat starch/WPI mixtures

Samples	σ_{max} (Pa)	γ_{max} (%)
Oat Starch	288.85±1.06^{c}	129.50±2.12^{d}
WPI 25%	167.50±1.98^{e}	83.23±1.25^{e}
WPI 50%	238.69±1.68^{d}	162.37±1.65^{c}
WPI 75%	377.60±0.84^{b}	236.66±2.06^{b}
WPI 100%	1036.11±7.23^{a}	289.17±2.09^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

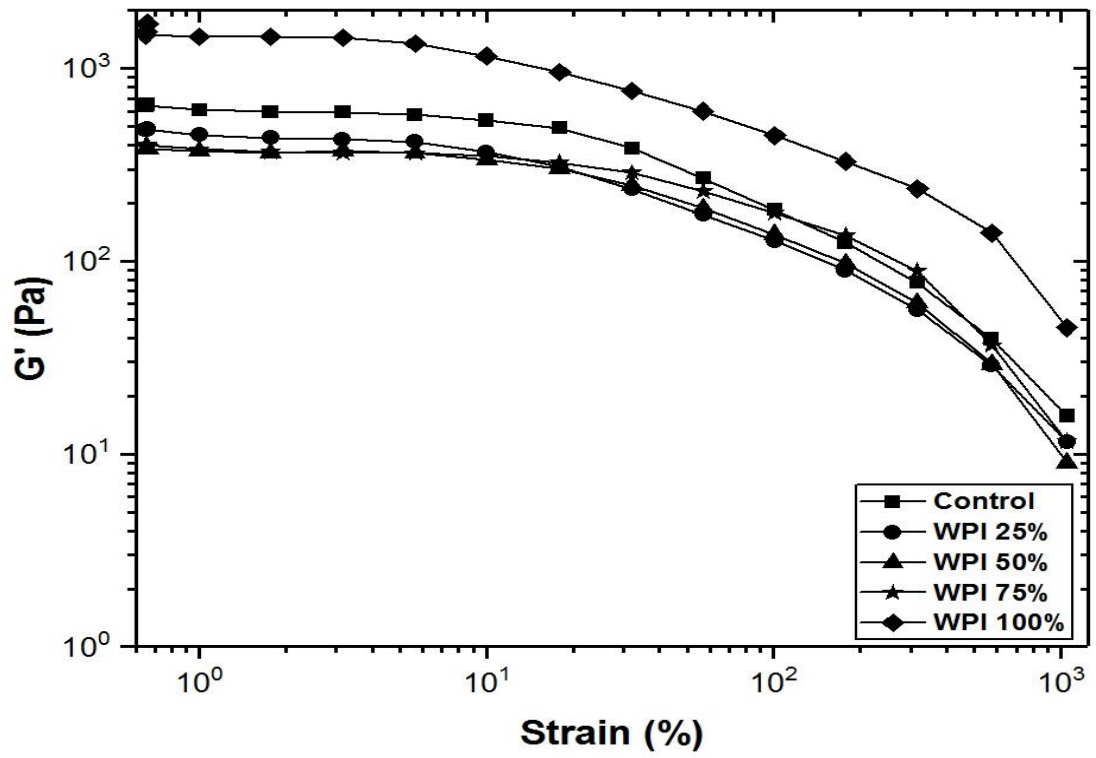


Figure 8-3 Elastic modulus (G') values as a function with increasing strain (%) in oat starch/WPI gels

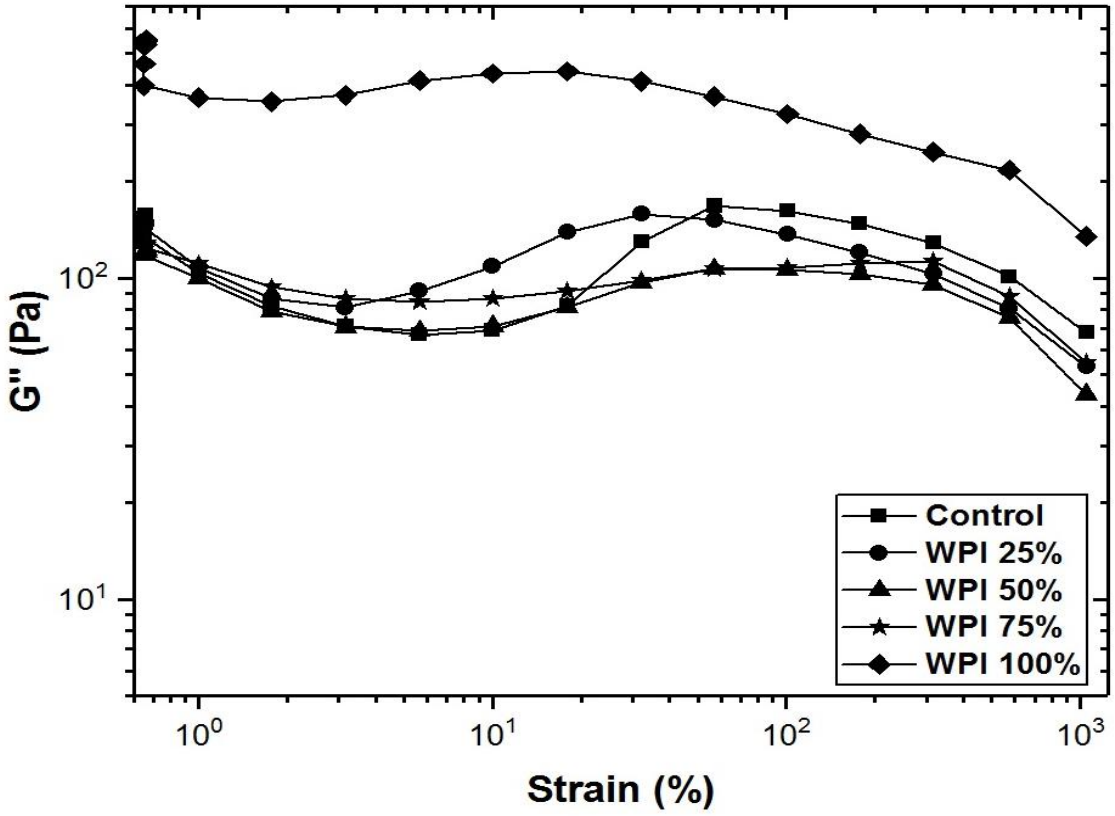


Figure 8-4 Viscous modulus (G") values as a function with increasing strain (%) in oat starch/WPI gels

The γ_{max} for oat starch alone was found to be 129 % (Table 8.3). The addition of 25% WPI decreased the γ_{max} significantly in comparison with oat starch alone but increased at higher concentrations and was maximum at WPI 100% (289 %). The σ_{max} also showed the same trend. Shim and Mulvaney (2001) also reported that at low level of WPI the fracture strain was constant and increased as WPI concentration was increased. Noisuwan et al. (2009) also reported the similar trend in rice starch/WPI gels.

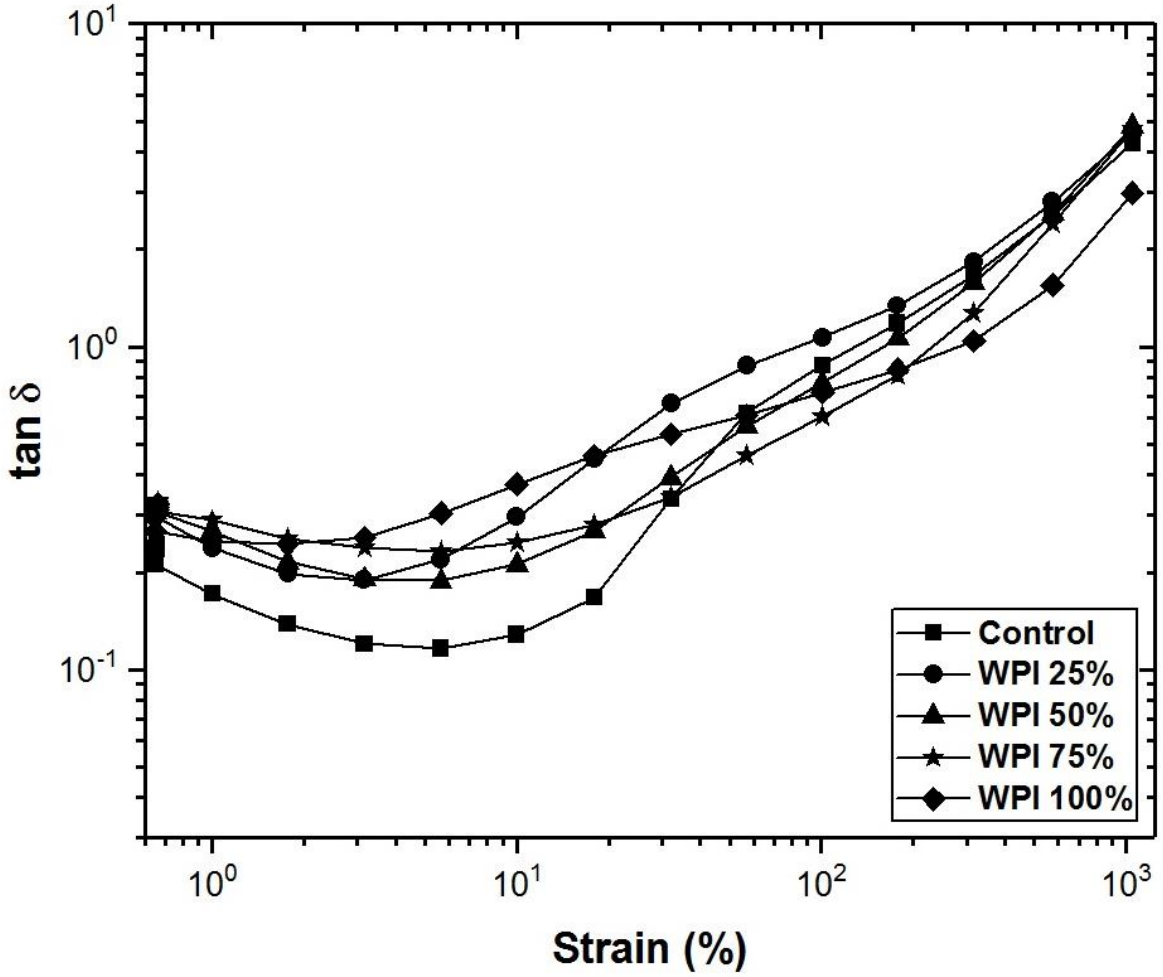


Figure 8-5 Damping factor (tan δ) as a function with increasing strain (%) in oat starch/WPI gels

Doublier (1994) suggested that the continous phase composed of leached starch molecules, and interactions between the continuous phase and the present protein in the matrix can impact the pasting of starch and their rheological properties. Pasting of starch in different continuous matrix impacts granule swelling behavior, which was discussed in previous chapter

4 and 6. As the pasting continues swollen starch granules ruptures and amylose and amylopectin comes out in continuous matrix and the protein present in that matrix could affect the rheological behaviour of cooled gels via many hydrophilic groups such as amide, hydroxyl, caroxyl and thiol in the alkyl side chains, which are capable of forming links with starch molecules (Goel et al., 1999). A study conducted by Yang et al. (2013) also supported on covalent hydrophobic bonding between wheat starch and WPI cooled gels.

8.3.2 Thixotropy analysis

The thixotropic behaviour of oat starch gels was previously studied by Doublier, Paton, and Llamas (1987) and proved to be more thixotropic in nature than wheat and maize starch. To understand this thixotropic nature of oat starch gels with WPI composites gels, three methods were employed: hysteresis loop, three interval thixotropy test and time dependent flow behaviour. The measurements of shear stress versus shear rate for increasing and decreasing curves showed a hysteresis loop or area indicating thixotropic and time dependent

Table 8.4 Three interval thixotropy and hysteresis loop test parameters of oat starch/WPI gels

Samples	Rec_{60} (%)	η_o (Pa.s)	η (Pa.s)	$\Delta\eta$ (Pa.s)	Hys Area ($Pa.s^{-1}$)
Oat Starch	66.07±1.04^{a}	140.61±3.39^{c}	91.95±1.77^{b}	48.66±1.62^{c}	4879.6±82.7^{b}
WPI 25%	67.00±1.14^{a}	136.31±0.97^{c}	86.91±0.12^{b}	49.40±0.84^{c}	4858.6±66.8^{b}
WPI 50%	55.26±0.57^{b}	139.05±1.48^{c}	70.78±1.59^{c}	68.27±3.08^{c}	4189.4±104.2^{c}
WPI 75%	44.16±0.54^{c}	195.87±3.02^{b}	87.81±0.82^{b}	108.05±2.19^{b}	3949.3±75.7^{c}
WPI 100%	18.82±1.38^{d}	2166.5±19.1^{a}	383.50±7.78^{a}	1783.00±11.31^{a}	8359±107.8^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

flow behaviour of samples (Figure 8.6) (Tárrega, Durán, & Costell, 2004). The area of hysteresis loop is a measure of extent of thixotropy (Razavi & Karazhiyan, 2009). Table8.4 showed the hysteresis area of oat starch alone and starch/WPI mixtures. The data showed that at low level WPI addition (25%) there was no significant difference in hysteresis area, while it decreased at 50% and 75% WPI addition in comparison with oat starch alone. Hysteresis area was maximum at 100% WPI addition to oat starch. The magnitude of hysteresis loop area represents the energy required for structural breakdown (Roopa & Bhattacharya, 2009). Therefore, up to 75% WPI addition, weaker gel behaviour was observed, and a strong thixotropic pattern exhibited by 100% WPI/starch gel. The results obtained by hysteresis analysis are following the trend of the steady state and large deformation strain sweep study results and indicated the weakening of starch gel in presence of WPI up to 75%.

Table 8.5 Weltman model and time dependent test parameters of oat starch/WPI gels

Samples	A (Pa)	-B (Pa)	R^2	η_o/η
Oat Starch	701.79±3.05^{b}	75.83±0.85^{b}	0.99	3.04±0.11^{b}
WPI 25%	586.35±3.00^{c}	64.84±0.38^{c}	0.98	2.77±0.43^{b}
WPI 50%	542.26±1.30^{e}	57.68±0.79^{d}	0.98	2.82±0.05^{b}
WPI 75%	575.53±1.16^{d}	52.95±0.11^{e}	0.96	2.73±0.48^{b}
WPI 100%	1138.34±2.98^{a}	83.08±1.20^{a}	0.95	8.40±1.90^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

The three interval thixotropy test also represented the thixotropic nature of oat starch gels

(Figure 8.7). Recovery percentage (Rec_{60}**)** after 60 sec was calculated after deformation along with initial (η_o), final (η) and the change ($\Delta\eta$) in viscosities (Table 8.4). Recovery percentage decreased significantly above 25% WPI addition in comparison with oat starch alone, and only 18% structure recovery was observed for 100% WPI/starch gels. Initial (η_o), final (η) and the change ($\Delta\eta$) in viscosities also represented the weak thixotropic nature of composites in comparison with starch alone.

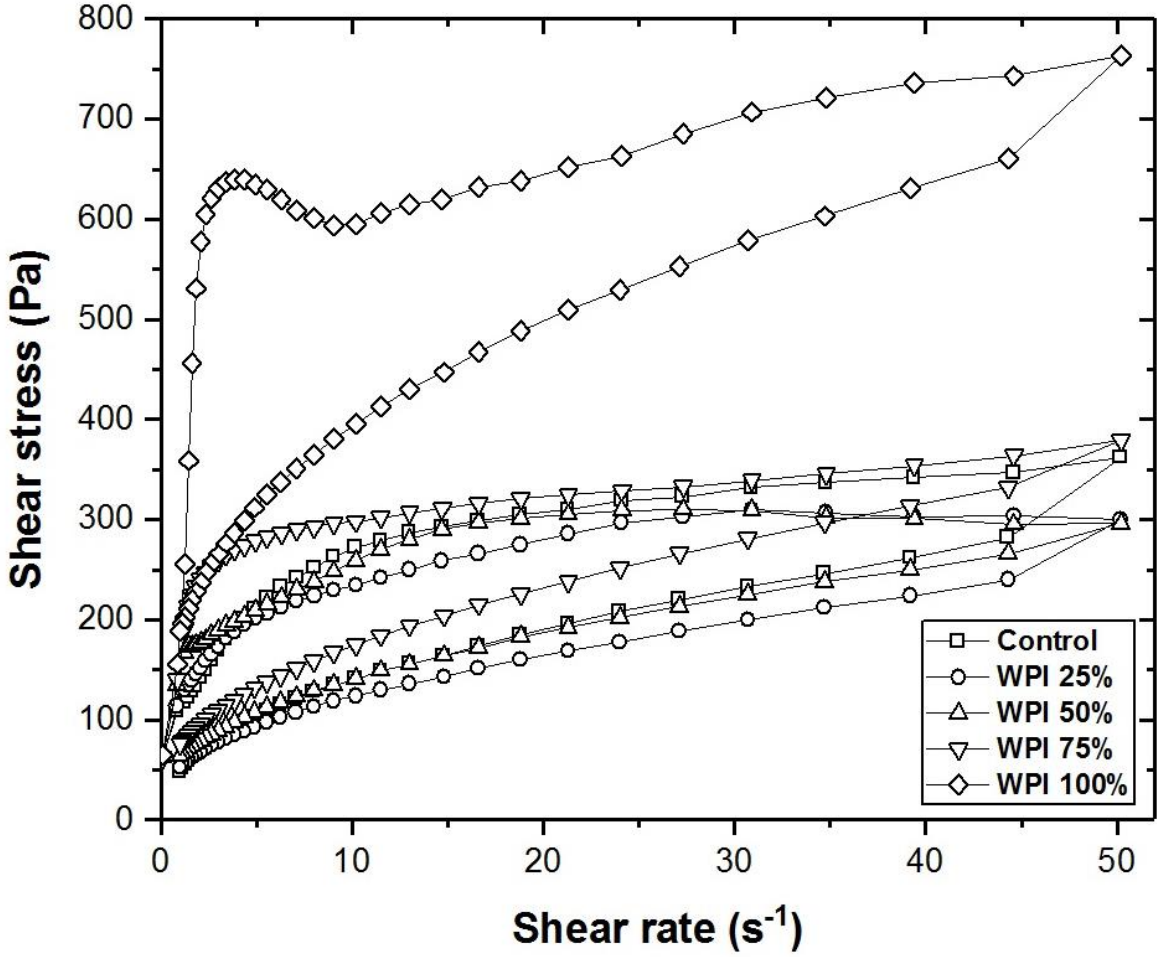


Figure 8-6 Shear stress as a function of increasing and decreasing shear rate in hysteresis loop test of oat starch/WPI gels

Thixotropic behaviour is highly dependent on microstructure and depends upon shear, and it is usually due to weak hydrophobic or attractive forces between particles. The molecules tend to move in shear direction during shearing forces and the intermolecular weak bonds incline to be broken easily depending on gradient (Mewis & Wagner, 2009). The addition of WPI in

system, helped in weakening the reversible intermolecular bonding between leached amylose molecules, however at 100% WPI addition, the gelling capacity of WPI increased the energy requirement to break the structure which is discussed earlier.

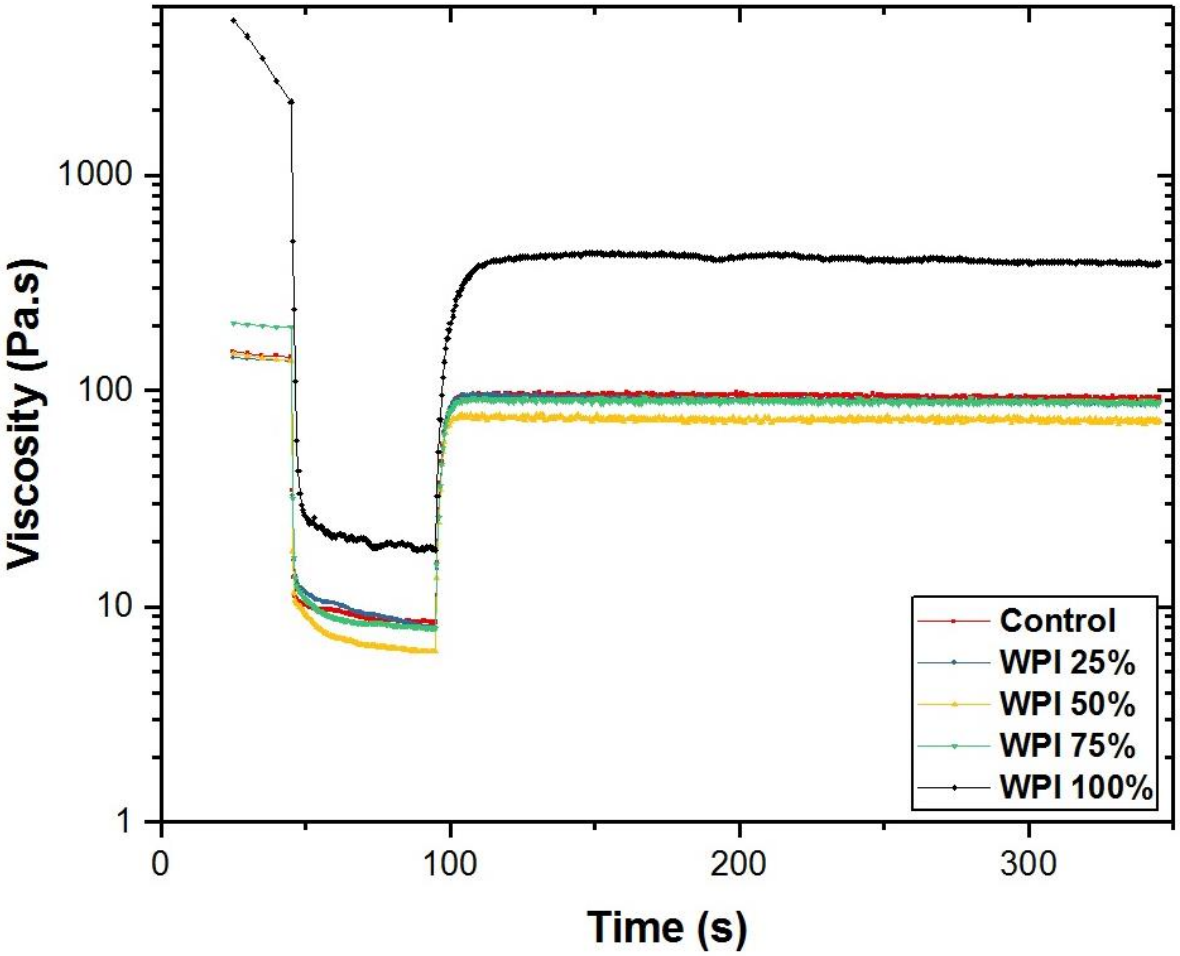


Figure 8-7 Viscosity values as a function of time and shear in oat starch/WPI gels (Three interval thixotropy test)

The time dependent flow properties of oat starch and starch/WPI composites were also measured by shearing the samples at constant shear rate 50 s^{-1} for 5 minutes. The rate and extent of shear stress decay over the time is represented in Figure 8-6. The Weltman model indicates the logarithmic change in shear stress with shearing time, parameter A represented the shear stress needed for structural breakdown and parameter B indicating quantitative structural damage during shearing (Dolores Alvarez & Canet, 2013). The values of Weltman model parameters A and B are presented in Table 8.5 along with η_o/η. R^2 values (0.95–0.99)

showed a good fit of Weltman model to experimental data (Table 8.5). The ratio of initial to final viscosity, η_o/η can be a relative measure of the amount of relative structural breakdown or extent of thixotropy (Abu-Jdayil, Al-Malah, & Asoud, 2002; Razavi & Karazhiyan, 2009). The η_o/η significantly increased at 100% WPI/starch mixture, which suggested increase in thixotropy.

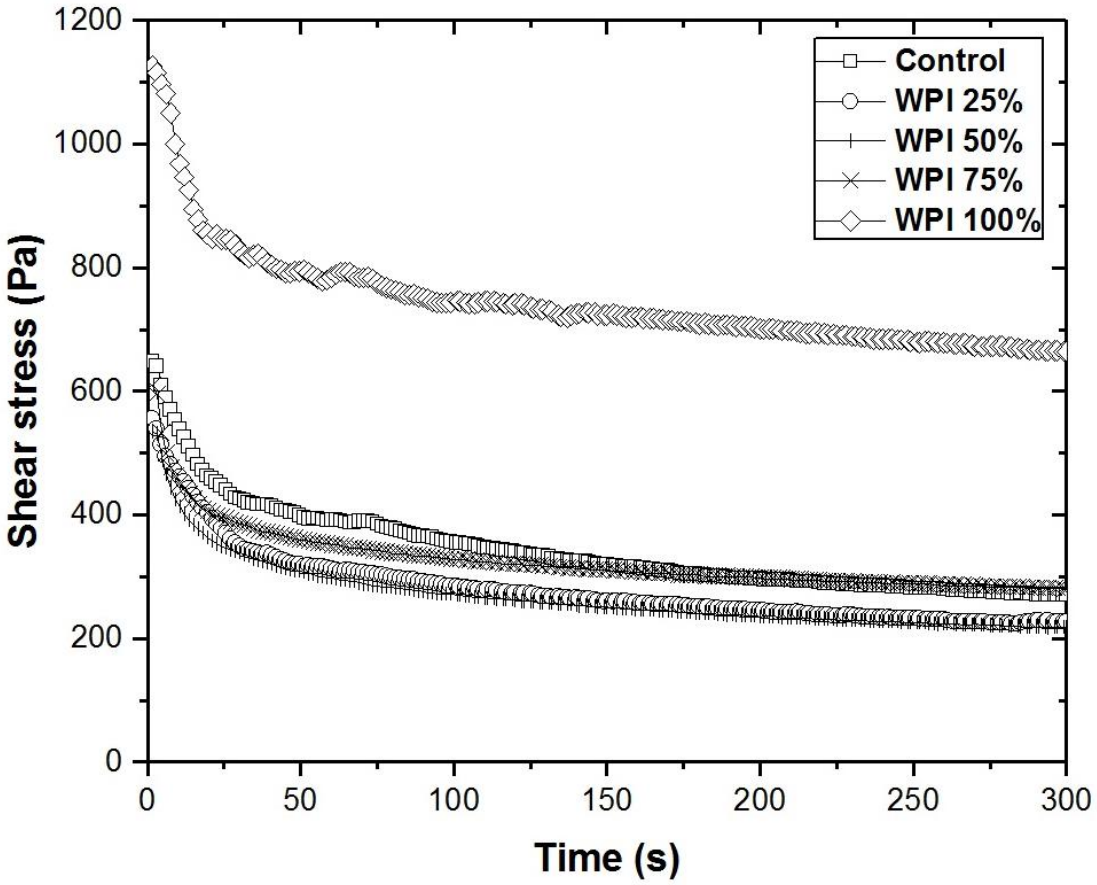


Figure 8-8 Time dependent flow behaviour of oat starch/WPI gels

The parameter A, which represented the initial shear stress, decreased significantly upon addition of WPI up to 75% level in comparison with oat starch alone. whilst, showed an increase at 100% WPI addition. The values of B also decreased significantly by the addition of WPI and relative to increased concentration up to 75% WPI, and then increased at 100% oat starch/WPI mixture. The negative values of B represents the decay of initial shear stress in relation to time coefficient to the steady state or final value (Koocheki & Razavi, 2009), hence

suggested that the degree of thixotropy of WPI/starch mixtures reduced upon addition of WPI, while increased at high concentration of WPI. The results obtained by time dependent studies are following the trend observed in the hysteresis loop and three interval thixotropy test study results.

8.3.3 Textural properties

Textural properties studies can be correlated to stress-strain sweep studies to reveal the sensory properties of food products. Penetration textural tests follow the disruption of gel network and could mimic the chewing in mouth or food processing. The textural parameters, i.e., elasticity, hardness, brittleness and adhesiveness are reported in Table 8.6. Elasticity is defined as the gradient or slope at the initial linear region of the curve, which significantly ($p<0.05$) decreased in the samples where WPI is added in oat starch up to the level of 75%. There was no significant difference observed in 100% WPI/starch gel in comparison with oat starch. The textural penetration test parameter, “Elasticity” had shown similar trend as observed by G’ (elastic modulus) in amplitude strain sweep experiment, which is discussed earlier in this chapter. Brittleness is the distance to peak force, increased significantly only at addition of high concentration (75% and 100%) of WPI in oat starch gels. Brittleness is highly dependent upon junction zone materials and concentration of different polymers present in a gel system, integrity and extent of interaction between polymers also plays an important role in fracture properties (Oates, Lucas, & Lee, 1993).

Table 8.6 Textural parameters of oat starch/WPI gels

Samples	Elasticity* (N.s)	brittleness* (mm)	Hardness* (N) Gel strength	Adhesiveness (N)
Oat Starch	0.039±0.05^{a}	7.086±0.595^{c}	0.346±0.048^{b}	-0.107±0.012^{ab}
WPI 25%	0.031±0.039^{bc}	7.370±1.435^{c}	0.248±0.022^{c}	-0.084±0.016^{a}
WPI 50%	0.029±0.026^{cd}	8.094±1.766^{bc}	0.249±0.026^{c}	-0.093±0.015^{ab}
WPI 75%	0.024±0.027^{d}	9.660±0.630^{ab}	0.279±0.014^{bc}	-0.116±0.007^{b}
WPI 100%	0.036±0.051^{ab}	9.989±0.595^{a}	0.585±0.118^{a}	-0.096±0.040^{ab}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

WPI at low concentration provided not enough non-junction zones so that a crack can propagate through gels more easily, however at 75% and 100% WPI addition, the greater number of these zones increased the brittleness. Hardness is defined as the peak force observed during the compression cycle, which significantly decreased upon addition of WPI up to level of 75% to oat starch, whilst showed an increase at 100% WPI addition. Hardness can be correlated to G* (complex modulus), which is a measure of stiffness or rigidity, both parameters showed the same trend. Whey proteins has higher tendency to form whey aggregates and gel, which can contribute to hardness of oat starch gel at high concentrations (100%). The negative force produced by the return of probe is defined as adhesiveness, seemed not to be affected significantly by addition of WPI in oat starch, which could be due to intermolecular bonding of protein chains by aggregation.

8.4 Conclusion

The rheological study of oat starch gels and WPI composites comprised of steady state flow behaviour, amplitude strain sweep, thixotropy analysis and time dependent properties. It was found that oat starch-based gels exhibited shear thinning and thixotropic behaviour, and it increased upon addition of whey proteins in the system. Both power law and H-B model fitted well to the data. Amplitude sweep studies revealed that G' and G* decreased upon addition of WPI up to the 75% level and increased at 100% WPI. Hysteresis loop area also decreased in the same way, and increased at 100% WPI, representing a prominent effect on thixotropy. Both Weltman model parameters, A and B decreased and increased, respectively, at 25%, 50%, 75% and 100% WPI addition. Three interval thixotropic test, which is a novel technique to assess thixotropic nature of food gels, supported the other thixotropic tests. Textural penetration studies also supported the other rheological tests such as, amplitude strain sweep analysis. In the end, it can be concluded that WPI addition upto 75%, decreased the rheological parameters, suggesting weakening of oat starch gel and transformation from viscoelastic solid to viscoelastic liquid gels, which is due to WPI effect on starch gelatinisation by restricting swelling and availability of water. However, at 100% WPI concentration, the capacity of whey protein aggregation and gelling ability resulted in stiff gels (evident from increase in G', G* and hardness).

Chapter 9

9 Rheological and textural characteristics of oat starch-caseinate composite gels

9.1 Introduction

In previous chapter, WPI effect on rheological characteristic of oat starch gel was investigated, WPI showed a prominent effect on oat starch gels. To further illustrate the effect of other milk proteins such as caseins in the form of CaCN, which makes 80% of total milk protein, is studied to understand the rheological compatibility between CaCN and oat starch.

Rheological properties of starch pastes such as viscosity, texture, heat and shear stability and retrogradation tendency play an important role in industrial applications as thickeners, stabilizers or as gelling agents (Bemiller, 2011). Kelly *et al.* (1995) studied sodium caseinate effect on potato and maize starch, They reported that even a very small concentration of sodium caseinates (0.01%) resulted in a decrease in starch paste viscosity, while the maximum effect was seen at 1% concentration. Bertolini *et al.(*2005) reported increase in storage modulus (*G'*) in all the starches when sodium caseinate is added except in the case of potato starch , where it decreased. The changes occur during steady state rheology, which represents as function of shear rate, need rheological equations such as Herschel–Bulkley or power law to fully understand them. Amplitude strain sweep or large viscoelastic deformation tests helps to understand the rheological behaviour of food under large forces such as chewing or mouthfeel (Razavi & Karazhiyan, 2009). Furthermore, certain materials not only depend upon shear rate but also get affected by change in time, which comes under the thixotropy and time dependent materials (Abu-Jdayil et al., 2004; Wiktor Berski et al., 2016). Some of the methods used to detect this phenomenon are hysteresis loop test, three interval thixotropy test and time dependent flow test.

Although extensive literature is available on the rheology of mixed caseinates/caseins and various starch dispersions, there do not seem to be much information on calcium caseinate and oat starch dispersions, especially whether they exhibit synergistic or antagonistic rheological behaviour. Therefore, an attempt was made to elucidate the rheological and textural properties of CaCN and oat starch mixtures by using steady, dynamic, time dependent, three interval thixotropic and hysteresis loop tests.

9.2 Material and Methods

9.2.1 Materials

This section is described in 3.1

9.2.2 Sample preparation

The mixtures with oat starch and calcium caseinate (CaCn) were prepared as 1:0, 1:0.25, 1:0.5, 1:0.75 and 1:1 and starch concentration was kept constant 10 % (w/w) for all the experiments. Gel samples were prepared as described in pasting section and then various rheological test were performed at 25 °C.

9.2.3 Rheological properties

9.2.3.1 Steady state and dynamic rheology

This section is described in 3.2.18.1

9.2.3.2 Thixotropy analysis

This section is described in 3.2.18.2

9.2.3.2.1 Hysteresis test

This section is described in 3.2.18.2.1

9.2.3.2.2 Three interval thixotropy test (3ITT)

This section is described in 3.2.18.2.2

9.2.3.2.3 Time dependent

This section is described in 3.2.18.2.3

9.2.4 Textural properties

This section is described in 3.2.12.1

9.2.5 Statistical analysis

This section is described in 3.2.19

9.3 Results and Discussion

9.3.1 Steady state and dynamic rheology

The steady state rheology test, including increasing shear rate effect on shear stress was studied and the results are reported in Figure 9-1. The flow curves were well fitted by the Power Law and Herschel-Bulkley model and presented in Table 9.1. The flow behaviour index ($n<1$) suggested the shear thinning behaviour of oat starch gels, a lower flow index value indicates a high shear thinning or pseudoplastic behaviour of gels (Pongsawatmanit et al., 2006). The addition of the CaCN supported the shear thinning behaviour of starch gels.

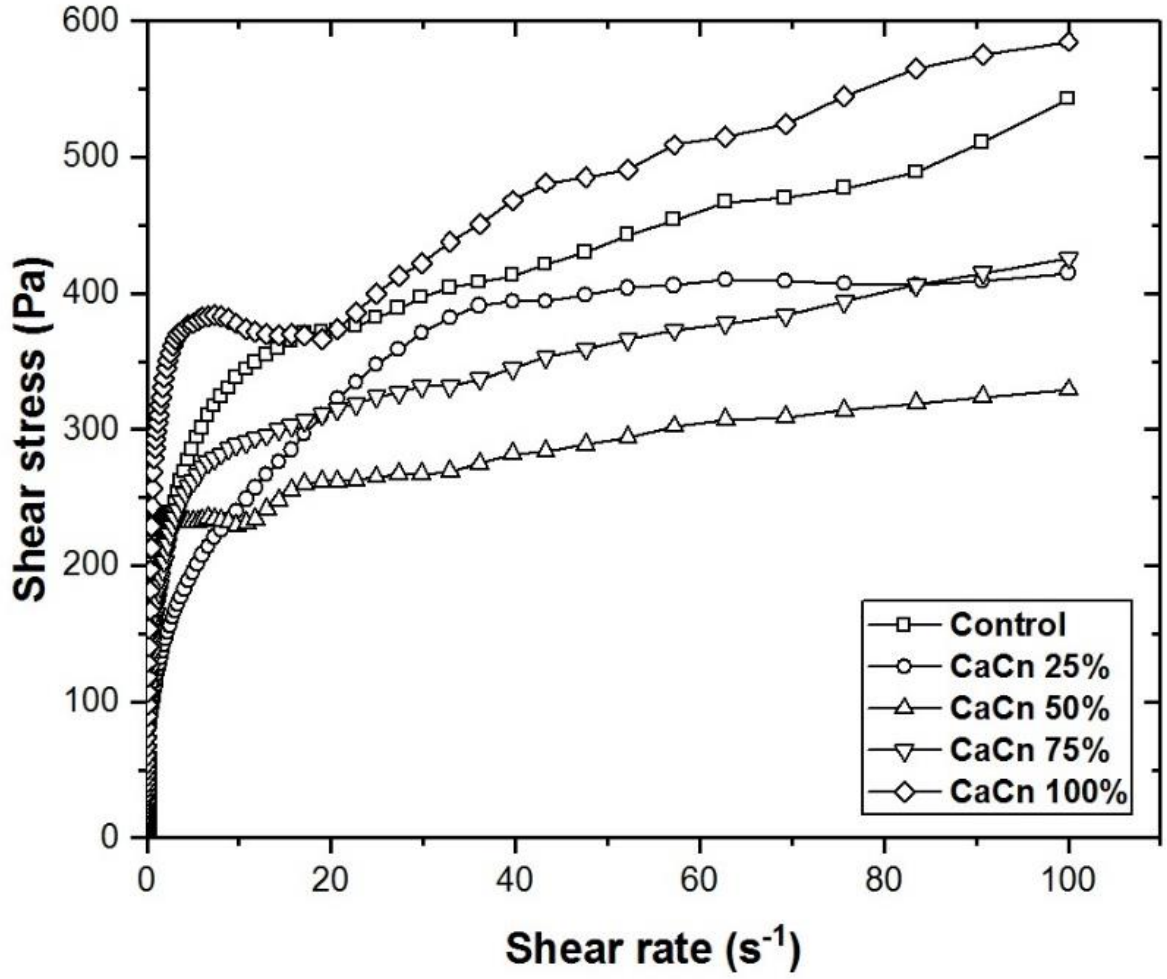


Figure 9-1 Shear stress values as a function of shear rate in oat starch/CaCN gels

As it can be illustrated from Table 9.1, Flow behaviour index (n) was significantly ($p < 0.05$) decreased upon addition of CaCN at 25%, 50% and 75% concentration, while remained unchanged at 100% in comparison with oat starch alone as obtained from both models. Consistency index (K) also showed a significant increase at 100% CaCN addition only by both the models. These results suggested that presence of CaCN is not affecting the consistency of gels, however resulting in a weak gel upto 75% addition. Lelièvre and Husbands (1989) examined the effect of sodium caseinate on corn and waxy maize starch and reported that addition of sodium caseinates increased the shear thinning. Similar results were also reported by Doublier, (1994) and Goel et al. (1999), where an increase in viscosity and decrease in flow index is observed in sodium caseinate-starch and casein corn starch systems respectively. In

this study, we observed only increase in viscosity at high concnetration of CaCN. Yield stress (σ_0) also decreased significantly upon addition of low concentration (25%, 50%) of CaCN, while increased at high concentration (75% and 100%). Kelly et al. (1995) also reported that even a very small concentration of sodium caseinates (0.01%) resulted in a decrease in starch paste viscosity.

Table 9.1 Steady state parameters of oat starch/CaCN mixtures

Samples	Power Law		H-B Model		
	n	K (Pa s^n)	n	K (Pa s^n)	σ_0 (Pa)
Oat Starch	0.214±0.004^{a}	189.67±0.77^{c}	0.181±0.009^{ab}	233.69±8.67^{c}	50.86±0.85^{d}
CaCN 25%	0.154±0.004^{c}	213.68±1.49^{b}	0.166±0.007^{bc}	132.38±4.14^{d}	30.05±0.28^{e}
CaCN 50%	0.117±0.004^{d}	188.14±1.33^{c}	0.112±0.004^{d}	226.13±2.07^{c}	64.72±0.84^{c}
CaCN 75%	0.174±0.007^{b}	181.53±1.61^{d}	0.145±0.004^{c}	268.61±6.03^{b}	89.86±0.94^{b}
CaCN 100%	0.191±0.002^{b}	235.56±0.91^{a}	0.202±0.004^{a}	603.46±5.17^{a}	133.00±1.78^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

Large deformation amplitude strain sweep tests were conducted on oat starch and composite gels, the resultant parameters are illustrated in Figure 9-2,9-3, and 9-4. The parameters obtained from strain sweep at 1% strain and at constant frequency of 1 Hz are illustrated in Table 9-2. As it can be illustrated from Table 9.2, at 1% amplitude, G' was higher than G", which represented viscoelastic solid gel nature. G' decreased significantly on addition of CaCN in oat

starch except for high concentration of 100%, where it increased predominantly. No significant difference was observed in G' at concentration 25% and 50%. G" had shown no change at low level (25%) of CaCN addition to oat starch, however, it significantly increased at 50%, 75% and 100% CaCN addition. The increase in G" suggested the increase in viscous nature of the gel (Figure 9.3).

Table 9.2 Amplitude sweep parameters of oat starch/CaCN gels at 1% strain and at constant frequency of 1 Hz

Samples	σ (Pa)	G' (Pa)	G" (Pa)	tan δ	G* (Pa)
Oat Starch	6.100±0.141^{b}	604±8.49^{b}	102.50±2.12^{c}	0.170±0.001^{e}	97.35±1.48^{b}
CaCN 25%	4.200±0.113^{c}	412.50±4.95^{cd}	99.00±2.83^{c}	0.242±0.002^{d}	65.20±4.10^{c}
CaCN 50%	4.550±0.042^{c}	431.50±2.12^{c}	147.00±1.41^{b}	0.340±0.000^{b}	71.50±1.98^{c}
CaCN 75%	4.190±0.014^{c}	395±1.41^{d}	144.50±3.54^{b}	0.362±0.001^{a}	66.950±0.354^{c}
CaCN 100%	7.255±0.219^{a}	705±5.66^{a}	210.50±3.54^{a}	0.305±0.007^{c}	114.50±4.95^{a}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

Complex modulus (G*), which indicates rigidity or stiffness, decreased significantly by the addition of CaCN in comparison with oat starch alone. However, no significant (p>0.05) difference was observed among 25%, 50% and 75% CaCN addition. The G* increased in 100% CaCN/ starch gels. A study carried out by Bertolini et al. (2005) on addition of sodium caseinate with different starches, they reported that the addition of sodium caseinate increased the storage modulus (*G'*) in all the starches except for potato starch, where it decreased. In our

study, we got the similar effects but only at high concentration of CaCN (100%), which is due to difference in native properties of sodium caseinate and CaCN.

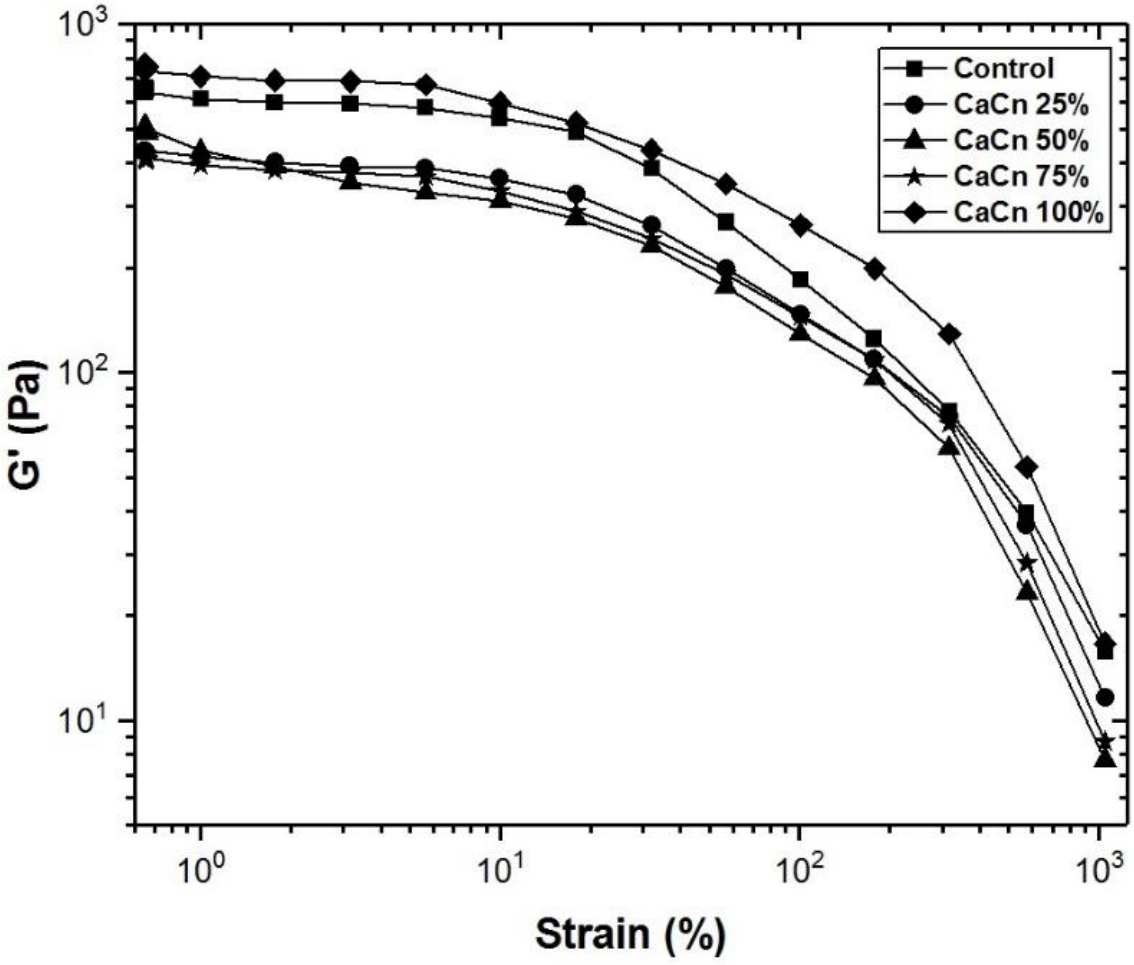


Figure 9-2 Elastic modulus (G') values as a function with increasing strain (%) in oat starch/CaCN gels

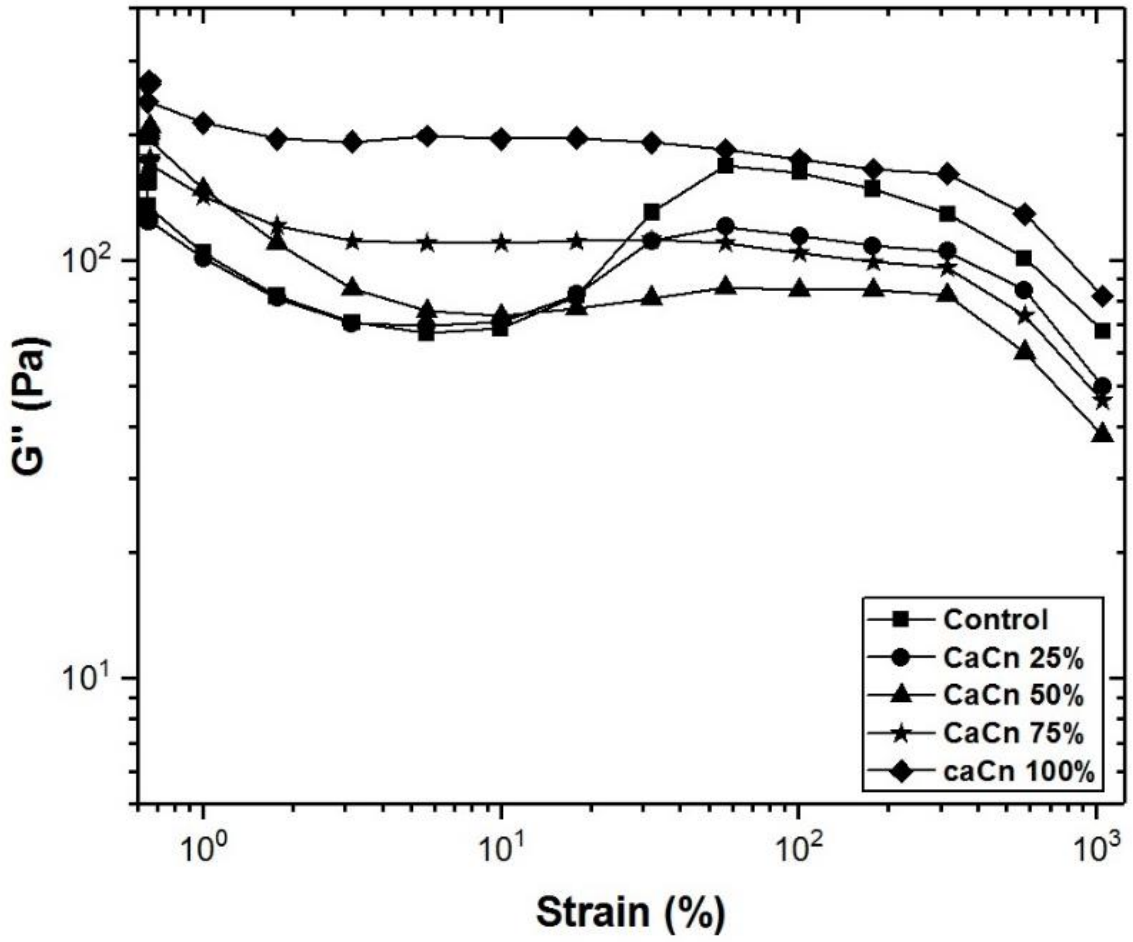


Figure 9-3 Viscous modulus (G") values as a function with increasing strain (%) in oat starch/CaCN gels

Noisuwan et al. (2009) studied the effect of sodium caseinate on rice starch, and they observed an increase in G* and G' upon addition of sodium caseinate. tan δ is a ratio of viscous modulus to elastic modulus, the values increased upon the addition of CaCN, indicated a viscoelastic liquid gel behaviour rather than viscoelastic solid gel (Figure 9-4). The increase in G", which is a viscous modulus also supported the weak gel behaviour of oat starch/CaCN gels.

Table 9.3 Flow point parameters of oat starch/CaCN gels

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

Samples	σ_{max}	γ_{max} (%)
Oat Starch	288.85±1.06^{b}	129.50±2.12^{d}
CaCN 25%	275.15±1.34^{c}	178.05±2.90^{c}
CaCN 50%	253.80±1.13^{d}	211.50±0.707^{b}
CaCN 75%	287.35±1.33^{b}	204.49±2.42^{b}
CaCN 100%	549.26±2.76^{a}	235.66±1.50^{a}

The flow point parameters are reported in Table 9.3, the maximum strain (γ_{max}) and maximum stress (σ_{max}) was calculated, which are defined as the strain and stress at a point where G'=G". The γ_{max} increased significantly (p>0.05) upon addition of CaCN in comparison with oat starch alone, in addition there is no significant difference observed among 50% and 75% CaCN gels. The σ_{max} decreased significantly up to 50% CaCN addition, remained same at 75% and increased again at 100% CaCN gels in comparison with oat starch alone. The findings in this study are contrary to the findings of Noisuwan et al. (2009), where the γ_{max} decreased upon addition of sodium caseinate in normal rice starch gels.

The reason behind the different results from past studies, were due to the unique behaviour of CaCN. CaCN showed a less weakening effect on the starch matrix in comparison with WPI. The results obtained by CaCN/oat starch gels are contrary to the previous studies, which were done on the sodium caseinate. CaCN has less solubility upon heating, more capacity to form large aggregates due to presence of calcium ions at low temperature, less heat stability and could form thin viscous gels in comparison with sodium caseinate, where sodium caseinate have a good capability of gelling, high solubility and heat stability (Singh, 2011; Srinivasan et al., 1999).

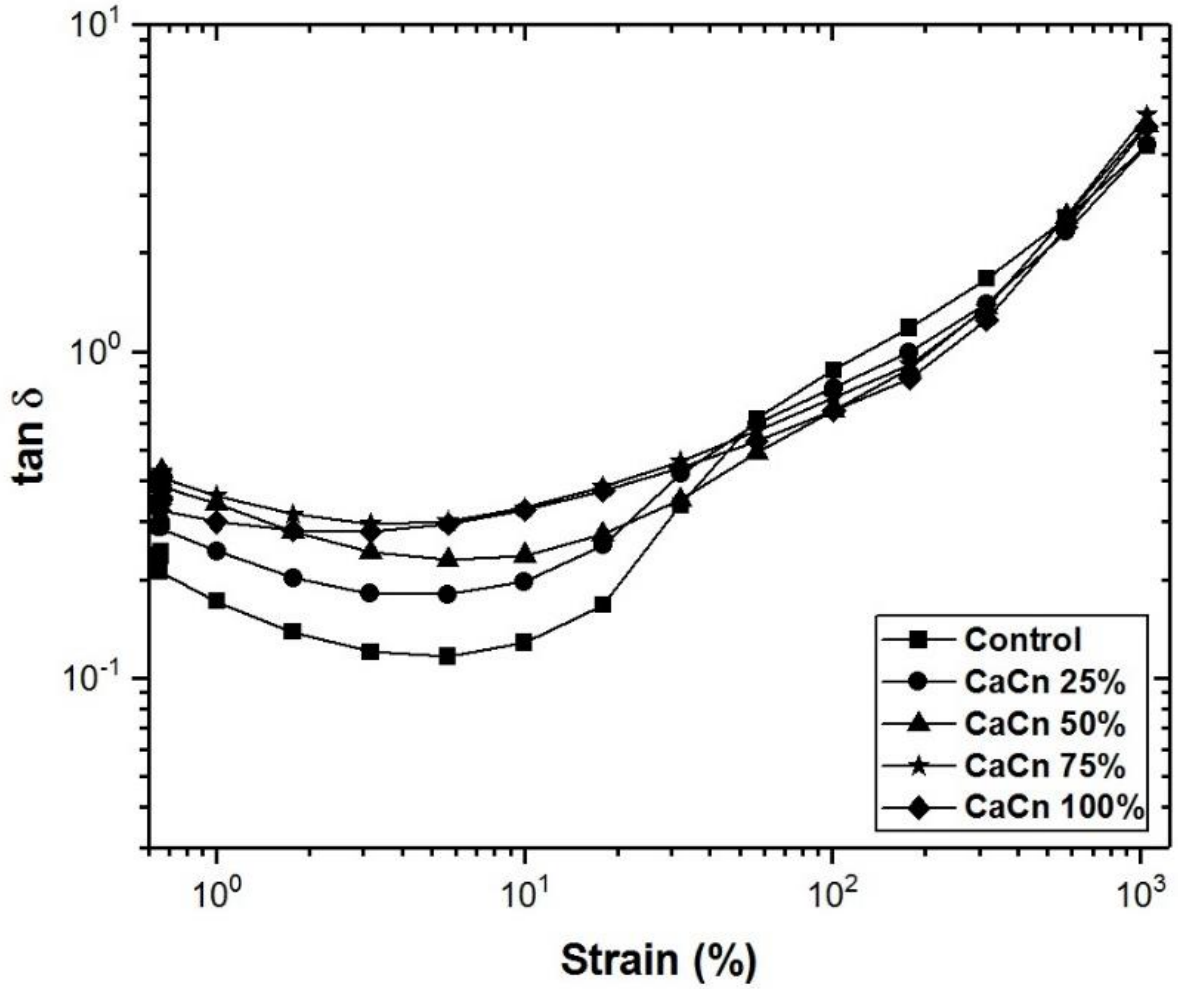


Figure 9-4 Damping factor (tan δ) as a function with increasing strain (%) in oat starch/CaCN gels

9.3.2 Thixotropy analysis

The thixotropic nature of oat starch gels with CaCN composites gels was studied by three methods namely hysteresis loop, three interval thixotropy test and time dependent flow behaviour. The measurement of shear stress when shear rate was increased from 1 to 50 s^{-1} and decreased from 50 to 1 s^{-1} showed a hysteresis loop, area was calculated and indicated the thixotropic behaviour of starch gels (Figure 9-5). The area of hysteresis loop is a measure of extent of thixotropy (Razavi & Karazhiyan, 2009).

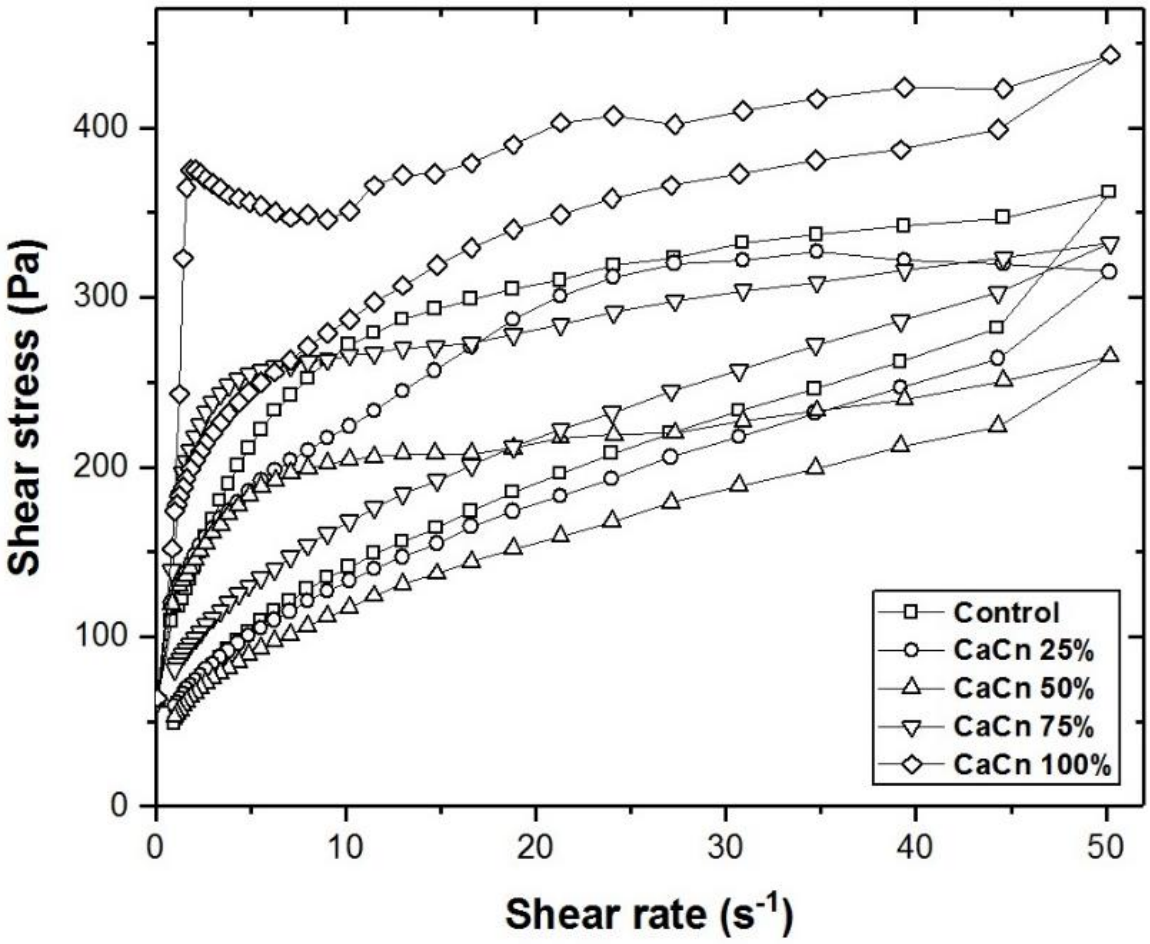


Figure 9-5 Shear stress as a function of increasing and decreasing shear rate in hysteresis loop test of oat starch/CaCN gels

As can be observed from Table 9.4, the hysteresis loop area was maximum (4879.6 Pa) for oat starch gel and decreased significantly upon addition of CaCN. The hysteresis area was found to be lowest at 50% CaCN/oat starch mixture. There was no significant difference observed among at 75% and 100% CaCn/starch gels. As CaCN concentration is increased, the energy required for the structural breakdown is decreased in all samples. It should be noted that thixotropy is also dependent on structural integrity, addition of CaCN increased the G" (viscous modulus) and decreased the G' (elastic modulus), which is described in previous section, therefore the energy required for structure breakdown decreased, and gel was transformed in more viscoelastic liquid state. Singh (2011) also reported that CaCN at high concentration tends to form reversible gels, which when cooled, can turn into viscous liquid rather than

elastic gels.

Table 9.4 Three interval thixotropy and hysteresis loop test parameters of oat starch/CaCN gels

Samples	Rec_{60} (%)	η_o (Pa.s)	η (Pa.s)	Δη (Pa.s)	Hys Area (Pa s^{-1})
Oat Starch	66.07±1.04^{a}	140.61±3.39^{c}	91.95±1.77^{b}	48.66±1.62^{c}	4879.6±82.7^{a}
CaCN 25%	65.09±1.16^{a}	111.10±2.97^{d}	66.82±0.735^{c}	44.28±2.23^{c}	4465.4±46.2^{b}
CaCN 50%	56.15±0.96^{bc}	108.26±1.78^{d}	60.30±0.56^{c}	47.96±1.21^{c}	2710.9±17.1^{d}
CaCN 75%	53.96±0.32^{c}	169.19±2.55^{b}	92.44±2.20^{b}	76.75±0.35^{b}	3028±79.1^{c}
CaCN 100%	59.90±1.10^{b}	273.00±5.66^{a}	166.50±3.54^{a}	106.50±2.12^{a}	2969.6±48.5^{c}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at $p<0.05$**

Three interval thixotropy test is employed and first, shear rate was kept constant at 1 s^{-1}, then gel structure breakdown occurred by ramping shear rate to 100 s^{-1} and in the final phase, shear rate kept constant at 1 s^{-1} (Figure 9-6). The structure recovery after 60 sec was calculated and termed as recovery percentage (Rec_{60}). Initial (η_o), final (η) and the change (Δη) in viscosities were also calculated and presented in Table 9.4. The recovery percentage (Rec_{60}) was not affected at low level of CaCN addition (25%), above 25% CaCN, the effect of CaCN was not constant, Rec_{60} decreased significantly at 75% CaCN, increasing again at 100%, while 50% CaCN shared the structure recovery with both CaCN 75% and 100%. Initial (η_o), final (η) and the change (Δη) in viscosities also represented the significant weak thixotropic nature of composite gels only at 75% and 100% CaCN/starch gels. Doublier (1994) reported the sodium caseinate effect on wheat, potato and tapioca starches, they reported that addition of sodium

caseinate reduced the thixotropic character of the pastes. The reason behind this was given to lowering of starch molecules deformability and mechanical brittleness by presence of casein aggregates.

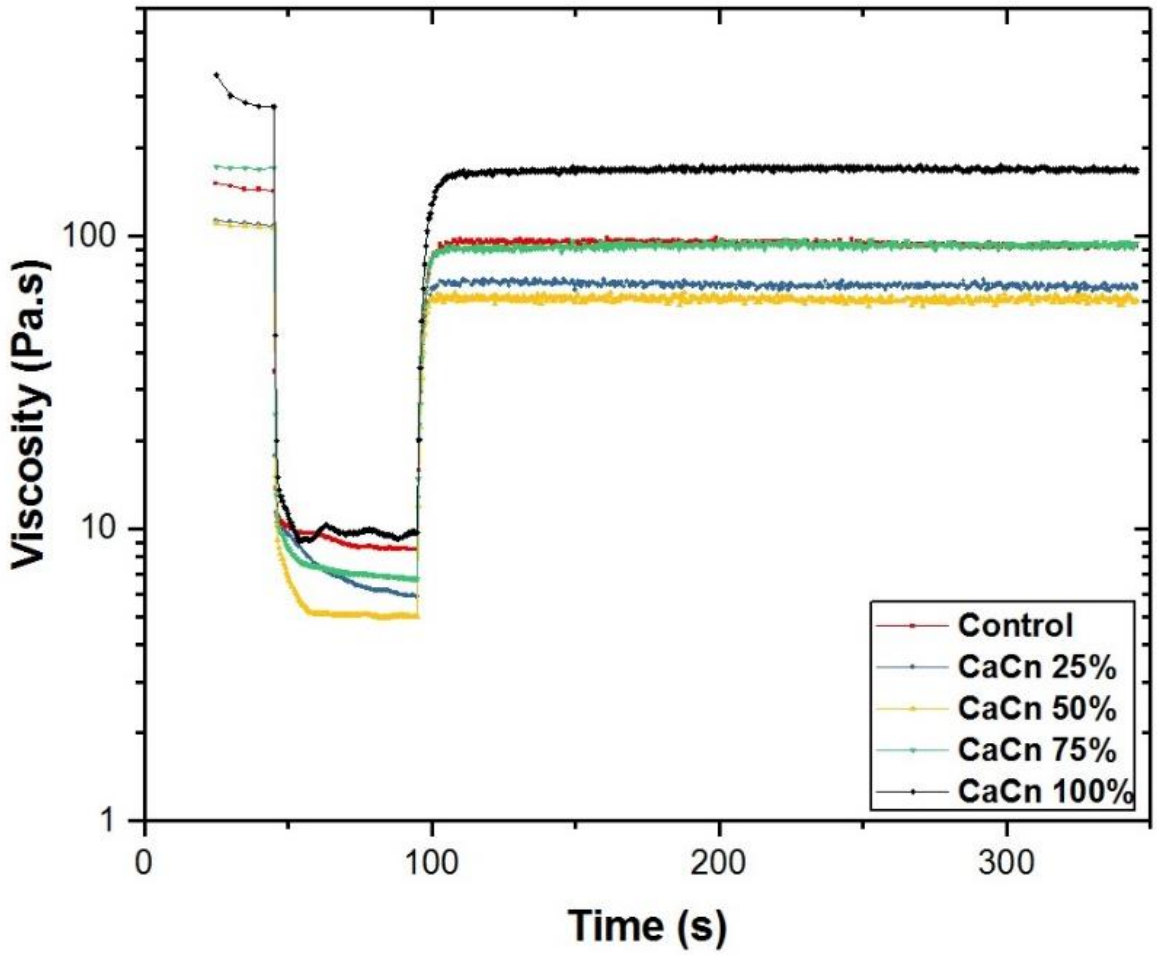


Figure 9-6 Viscosity values as a function of time and shear in oat starch/WPI gels (Three interval thixotropy test)

Time dependent flow properties of oat starch and starch/CaCN composites were also measured and the shear stress decay over the time graph is presented in Figure 9-7. The Weltman model parameters A, which is shear stress to initiate the structure breakdown and Parameter B, which is time coefficient of thixotropic breakdown are presented in Table 9.5. R^2 values (0.95-0.99) showed good fit of Weltman model to experimental data. The ratio of initial to final viscosity (η_o/η) represented the extent of thixotropy. The value of A decreased significantly when CaCN is added, the minimum value of A was observed at 75% CaCN/starch

gel. The values of B and η_o/η also decreased significantly in comparison with oat starch alone and found to be proportional to increasing CaCN concentration. The results supported the hysteresis loop test and three interval thixotropy analysis findings.

Table 9.5 Weltman model and time dependent test parameters of oat starch/CaCN gels

Samples	A (Pa)	-B (Pa)	R^2	η_o/η
Oat Starch	701.79±3.05[a]	75.83±0.85[e]	0.991	3.04±0.11[a]
CaCN 25%	524.51±3.20[c]	57.98±0.35[d]	0.985	2.78±0.10[b]
CaCN 50%	509.99±1.56[d]	49.62±0.69[c]	0.970	2.77±0.10[b]
CaCN 75%	483.01±0.48[e]	38.89±0.65[b]	0.953	2.32±0.08[c]
CaCN 100%	572.38±3.50[b]	33.01±0.21[a]	0.982	1.58±0.02[d]

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at $p<0.05$**

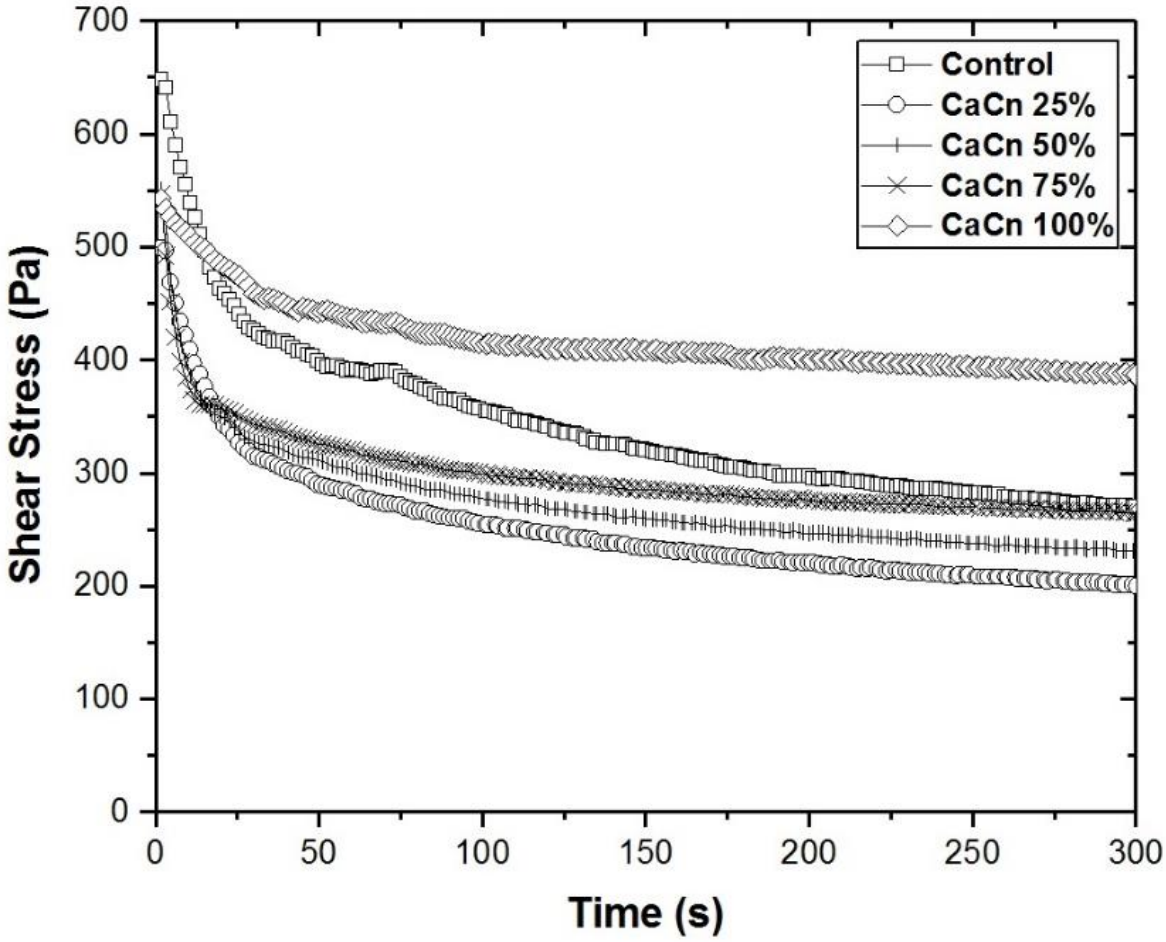


Figure 9-7 Time dependent flow behaviour of oat starch/WPI gels

9.3.3 Textural properties

The textural properties of different oat starch/CaCN gels were determined using texture analyser including elasticity (slope at the initial linear region of the curve), brittleness (distance to peak force), hardness (peak force) and adhesiveness (negative force during return of probe). The obtained textural parameters are reported in Table 9.6. Elasticity was decreased significantly upon addition of CaCN upto 75%, however, no significant difference was observed among 25%, 50% and 75% CaCN/starch gels. Both CaCN 25% and 50% significantly increased the brittleness in comparison with oat starch alone, with no difference among them. Same similar pattern was observed by 75% and 100% CaCN, the increase in brittleness is attributed to presence of non- junction zones, and weak gelling capacity by

CaCN. “Elasticity” had shown similar trend as observed by G’ (elastic modulus) in amplitude strain sweep experiment, which is discussed earlier in this chapter.

Table 9.6 Textural parameters of oat starch/CaCN gels

Samples	Elasticity* (N.s)	brittleness* (mm)	Hardness* (N) Gel strength	Adhesiveness (N)
Oat Starch	0.039±0.005^{a}	7.086±0.595^{c}	0.346±0.048^{b}	-0.107±0.012^{b}
CaCN 25%	0.020±0.003^{b}	8.118±1.048^{b}	0.228±0.012^{c}	-0.075±0.011^{a}
CaCN 50%	0.017±0.001^{b}	8.498±0.688^{b}	0.210±0.028^{c}	-0.089±0.012^{ab}
CaCN 75%	0.019±0.002^{b}	9.971±0.020^{a}	0.322±0.042^{b}	-0.076±0.021^{a}
CaCN 100%	0.035±0.002^{a}	9.987±0.015^{a}	0.490±0.052^{a}	-0.177±0.026^{c}

***Mean ± Standard deviation in the same column followed by different superscript differ significantly at p<0.05**

Hardness first decreased significantly upon the addition of CaCN upto 50% and increased at 100% CaCN/starch gel. Hardness can be correlated to G* (complex modulus), which is a measure of stiffness or rigidity, both parameters showed the same trend as discussed in previous sections. Similar results were reported by Wiktor Berski et al. (2016), where oat flour hardness increased in presence of sugars. In our case, CaCn consist of calcium ions, which can have same effect as sugars.

9.4 Conclusion

The rheological study of oat starch gels and CaCN composites comprised of steady state flow behaviour, amplitude strain sweep, thixotropy analysis and time dependent properties. Shear thinning behaviour of native oat starch gels increased significantly upon addition of CaCN.

Amplitude sweep studies revealed that G' decreased upto 75% level of CaCN and increased at 100%, while G" (viscous modulus) increased upon addition of CaCN. This pattern suggested that gels became weaker and more viscous upon addition of CaCN, because CaCN has a property to make viscous gels upon cooling. Thixotropy analysis studied by hysteresis loop test, three interval and time dependent test revealed that thixotropy decreased upon addition of CaCn. Textural penetration studies also supported the other rheological tests. In the end, it can be concluded that CaCN had an effect on oat starch, but it is not prominent as WPI. The CaCN addition with oat starch resulted in weak, viscous, brittle gels and indirectly showed that CaCN has less effect on starch properties in comparison with WPI.

Chapter 10

10 General conclusions and future recommendations

10.1 General conclusions

In the course of this study, the effect of milk protein ingredients on oat starch behaviour and its properties were investigated. The thesis was aimed into two main aspects, First, the extraction of oat starch from oat flour and effect of readily available commercial milk protein ingredients (WPC, WLAC and SMP) on oat starch and oat flour systems. Second, the effects of more pure fractions of milk proteins (WPI and CaCN) on oat starch in terms of thermal, pasting, structural and rheological properties.

The addition and substitution of milk protein ingredients to oat starch/flour resulted in different effects. The main findings of the study are highlighted below:

1. The first aspect of the study investigated the effects of substitution of oat starch with three different readily available milk protein ingredients (WPC, WLAC and SMP). The substitution of starch with milk protein ingredients decreased the native properties of oat starch system. The hot paste stability was increased when milk protein ingredients were used at 5% and 10% concnetration. Among all the ingredients, SMP showed a greater effect on starch characteristics in comparison with WPC and WLAC. SMP reduced the hot paste stability and viscosity, SMP and WPC both reduced the starch granule swelling and size and increased the freeze-thaw stability, this could have been due to presence of lactose, casein, salts and minerals present in SMP. Previous findings also reported that SMP had the resistant potential against the swelling of starch (Noisuwan et al., 2008, 2007). The results explained that oat starch dominates the continuous phase of starch gel and any substitution of starch will result in loss of textural properties of food, however, low-level (5%) substitution of starch with milk protein ingredients might help to improve

nutritional and textural quality except SMP. It was found that milk protein ingredients affected the oat starch in the order SMP>WPC>WLAC. **(Reported in chapter 4)**. Similar findings were observed in the study of substitution of oat flour with the milk protein ingredients. The native textural properties of oat flour were decreased as flour is substituted with milk protein ingredients, which supported the fact that continuous network (oat flour) is the key driving component in maintaining textural properties. SMP increased the pasting temperature in comparison with others suggesting resistance to swelling of starch molecules present in oat flour. However, no difference was observed among substituted milk protein ingredients with regards to syneresis and large deformation textural studies. The results obtained in this chapter followed the same trend as chapter 4, but the individual effect of different milk ingredients on oat flour was not dominant. This chapter revealed the oat flour complex behaviour as it included oat starch, lipids, protein and fibre, and the milk protein ingredient failed to demonstrate the same effect as in the case of oat starch. **(Reported in chapter 5)**

2. The second aspect of the study emphasis on the effect of the addition of different concentrations of pure protein fractions (WPI and CaCN) on oat starch. The increase in hot paste stability and decrease in relative breakdown was observed when WPI is added in the oat starch system. WPI also increased the peak temperature (T_p) and affected the thermal behaviour of oat starch as revealed by DSC studies. It is evident that WPI affected the gelatinisation of starch granules by restricting the swelling, which might be due to unavailability of water. The XRD and FTIR studies were conducted to understand long and short-range crystallinity order and revealed that WPI increased the ordered structure in Oat starch/WPI gels, as relative crystallinity increased while a decrease in IR bands at 1024 cm^{-1} and 1080 cm^{-1} supported less gelatinisation of starch molecules. **(Reported in**

chapter 6). Contrary to the WPI, CaCN, which consisted of large casein aggregates and loose caseins, had not shown prominent effect on oat starch at low concentrations (25% and 50% CaCN). Setback and stability ratio were decreased only at high concentrations (75% and 100%) in pasting analysis. A slight increase in T_p was observed, irrespective of increasing concentration of CaCN. XRD studies revealed no or little effect on crystallinity of oat starch, while increase in respective IR bands suggested amorphous structuration indicating less effect on gelatinisation. **(Reported in chapter 7).**

3. The rheological study of oat starch gels and their composites with WPI and CaCN were investigated in terms of steady state flow behaviour, amplitude strain sweep, thixotropic test and time dependent properties. WPI increased the shear thinning behaviour of oat starch gels as well as thixotropic nature. Elastic modulus (G') and complex modulus (G*) decreased upon addition of WPI upto 75% level and increased at high concentration (100%). It was concluded that addition of WPI upto 75% weakened the oat starch gel, probably due to its effect on starch gelatinisation in terms of reduced starch swelling. However, at high concentration (100%), the gelling ability of whey proteins resulted in stronger gels. **(Reported in chapter 8).** CaCN showed the same trend as observed by WPI in shear thinning behaviour of oat starch gels. While, viscous modulus (G") increased upon addition of CaCN resulting in a more viscous gels in comparison with WPI/starch gels. Thixotropy is also decreased by the CaCN. Finally, it can be concluded that CaCN had similar effect on oat starch gels as WPI, but it was not so prominent as WPI, CaCN resulted in weak, viscous and brittle gels. **(Reported in chapter 9)**

10.2 Correlation and summary

Throughout the work described in this thesis, several new findings were observed. Even though, extensive studies have been carried out on milk proteins with different starches (de

Bont et al., 2002; Fitzsimons et al., 2008; Kelly et al., 1995; Kett et al., 2012, 2013; Matignon et al., 2014; Matignon et al., 2015; Noisuwan et al., 2011; Noisuwan et al., 2008, 2007, 2009; Camilla Olsson et al., 2002; Sopade et al., 2006; Vu Dang et al., 2009), very limited research has been reported on oat starch-milk protein systems. Previous studies have shown that the biopolymers, WPI and sodium caseinate, have a prominent effect rice starch properties, where sodium caseinate affected the starch characteristics to a greater extent when compared with WPI (Noisuwan et al., 2008, 2007, 2009; Sopade et al., 2006). However, in this thesis, it was observed that WPI affected the oat starch properties to a greater extent in comaprsion with CaCN. WPI illustrated high potency to effect starch gelatinisation, possibly by competition between oat starch for water and aggregating behaviour upon heating. While, CaCN had shown less effect on starch gelatinisation, this might be due to less heat stability and gelling tendency of CaCN and less solubility upon heating. Starches are widely used in dairy-based food products, such as yoghurts, dairy desserts and processed cheese, Where they provide desired sensory and rheological properties (Oh et al., 2007; Zuo, Hemar, Hewitt, & Saunders, 2008). The substituition of oat starch with readily available commercial milk protein ingrediens (WPC, WLAC and SMP) suggested the usage of these ingredients in oat-dairy based food products such as oat meal, oat pudding and oat based sports drink.

The other acheivement of this work is that the most of the previous studies were done on addition of milk proteins, but here substitution of oat starch was prefered for the study. This is a novel approach to make food more nutritionally balanced in terms of reducing glycemic index. Both the milk proteins at high concentration (100%) showed an increase in rheological properties of oat starch gels in terms of rigidity and elasticity. The trends obtained from the resultant work could be used to predict the usage of oat starch/flour with milk protein ingredients in the development of novel food products and could elucidate fundamental

knowledge of starch-protein systems. In the thesis, it is evident that effect of milk proteins on oat starch is statistically extensive, this can be balanced by the optimisation of processing parameters such as availability of more water to reduce the effect of milk proteins on starch gelatinisation.

The thesis also indicates a range of questions and ideas for future investigations. Can the other casein forms such as acid casein, micellar casein have the same effect as CaCN? Does the botanical origin and physical surface of the starch affect the interactions between protein and starch? Does the pasting of oat starch in whole milk would have a dissimilar effect in comparison with the results we observed? These questions indicate the need of further research, but do not take away the importance of the work done in this thesis and the results presented for the use of milk protein ingredients in oat based systems.

10.3 Future recommendations

The current work has been taken to understand the fundamental and practical aspects of oat starch–milk protein ingredients interactions. Although lot of extensive work has been done in this study, some of the parts can be suggested for future work.

1. The present study was conducted on oat starch substituition and addtion with milk protein ingredients. Both the procedures affected the starch behaviour differently, however further research should be done by keeping the water content constant to observe the effects which is not due to limitation of water for gelatinisation.
2. Oat starch is a underutilized crop, very unique in terms of lipid content and small granule size, and it is a byproduct of β-glucan extraction from oat flour. Previously protein-starch interaction were studied in corn, wheat, rice and tapioca. This type of study should not be limited to coventional source of starches, the other pseudo-cereal starches such as

amaranthus and buckwheat should be investigated as they have also unique starch granule sizes.

3. This study is conducted on calcium caseinate (CaCN), which is chosen as an alternative to sodium caseinate, the effect of calcium in starch gelatinisation should also be studied to better understand the ions behaviour. Furthermore, starch digestibility studies should be done to understand the effect of milk proteins on digestibility.

11 References

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